EXHIBIT NO.

16

Parents accuse CDC of not reporting children's deaths from polio-like AFM

By Elizabeth Cohen, Senior Medical Correspondent
Updated 9:40 AM ET, Tue November 13, 2018

Moseley, Virginia (CNN) Parents of children who had a horrifying polio-like illness are accusing the Centers for Disease Control of hiding the deaths of two children who suffered from the condition.

The parents say by not publicly acknowledging the two deaths, the agency is intentionally downplaying the severity of acute flaccid myelitis (AFM), a disease that paralyzes healthy children in a matter of hours.

"I feel like they're just sugar-coating this," said Katie Bustamante, whose son Alex, age 6, died in May. "It eliminates my trust in the CDC."

Moms give CDC an 'F' for handling of polio-like illness 02:51

Their accusations come amid a wave of criticism from parents of children with AFM and from some of the CDC's own medical advisers. In a recent on-camera interview with CNN, a group of parents gave the agency an "F" for its handling of the outbreak.

A CDC official said while she couldn't comment directly on the boys' cases, there may be a "lag" in AFM reporting from physicians to health departments to the CDC.

"I think we want to catch up with the backlog," said Dr. Anne Schuchat, principal deputy director of the CDC, a 30-year veteran of the CDC and a retired rear admiral in the US Public Health Service. "Even the past week we've expanded the number of disease detectives on the program."

Schuchat, who twice served as acting director of the agency and helped lead the fight against pandemic flu, SARS and anthrax, said she was sorry to hear that the parents think the CDC is hiding something.

"I certainly want to make sure the information that we have is shared as quickly as possible," Schuchat said. "We wish we understood all that we need to about this disease

and how to best diagnose it, how to treat it and how to give families enough information about what to expect. I think it's very challenging when your child has been through something quite traumatic to not even know what the prognosis is."

She added that there's no simple lab test for AFM, so CDC disease detectives have to carefully review medical records.

Parents fear polio-like deaths underreported 02:43

Twenty-six states have confirmed cases of AFM, and 11 additional states have possible cases, according to a survey last week by CNN of state health departments. This year there have been 80 confirmed cases of the illness, and 219 cases are currently under investigation, according to the most recent CDC data.

On its AFM surveillance webpage, the CDC doesn't mention any deaths from AFM. At a press briefing last month, Dr. Nancy Messonnier, director of the CDC's National Center for Immunization and Respiratory Diseases, mentioned that the agency knew of one death in 2017, but did not mention any deaths this year, even when asked about it by a reporter.

Chris and Robin Roberts lost their 5-year old son, Carter, in September after a 2-year battle with AFM. CNN has seen portions of Carter's and Alex's medical records, which show their doctors had diagnosed them with AFM. In Carter's case, doctors at three medical centers -- Virginia Commonwealth University, Johns Hopkins and Boston Children's Hospital -- diagnosed him with the disease.

The CDC has set up a system where physicians report cases of AFM to their state health departments, which in turn report the cases to the CDC.

Two pediatric neurologists who serve as medical advisers to the CDC on AFM say they think the agency could be faster in reviewing and reporting cases and deaths.

"It shouldn't be taking this long to confirm these cases," said Dr. Keith Van Haren, assistant professor of neurology at the Stanford University School of Medicine and one of the CDC advisers. That kind of a turn around time for mortality reviews is a symptom of a disconnect at the CDC."

Carter's parents agree.

"They're doing a s*** job of measuring this, excuse my French," said his mother, Robin, a healthcare IT specialist.

Carter Roberts

'Mommy, mommy, help me'

On July 29, 2016, Carter vomited after dinner. The next day he had a fever of 99 degrees but was feeling well enough to eat and drink.

It's just a virus, thought his parents, who have two older children. It was nothing alarming.

The next morning, Robin went into her son's room and found him on the floor.

"Mommy, mommy, help me," his mother remembers him saying.

Robin remembers trying to help Carter stand up. His head flopped back. He couldn't use his right arm. She scooped up her son and took him to the emergency room.

Within a few days, Carter couldn't move anything below his neck. He was put on a ventilator and never came off.

For the next two years, his family and nurses did everything for him. They fed him. They scratched his nose when he had an itch. All day and all night they turned him every 90 minutes so he wouldn't get bedsores.

Carter was undeterred by his illness. He could read books by the time he was four. He instructed his father on how to construct characters out of Legos and medieval swords out of aluminum foil.

Carter went to pre-school, and in August of this year started kindergarten. He came home from his first day beaming about circle time and a pretty little girl in his class who wore a rainbow dress.

A few weeks later, on September 22, he was having trouble breathing. Robin was home alone with him and called an ambulance. His last words to her were: "Mommy, I'm fine."

Today, Carter's ashes sit in an urn in his family's living room.

Carter Roberts

A doctor wants answers

Both Carter's parents and Alex's parents say they're not sure if the CDC ever accepted their sons as official AFM cases back when they got sick in 2016.

Alex's mother says his doctor told her he reported his death to the California Department of Public Health, but she doesn't know what happened after that.

In Carter's case, his neurologist Dr. Sanjai Rao, told CNN he filled out paperwork on the CDC's website and following the CDC's instructions, sent the paperwork, lab specimens and MRI findings to the Virginia Department of Health.

An epidemiologist there confirmed Carter's information was sent along to the CDC, according to Rao, assistant professor of pediatric neurology at Children's Hospital of Richmond at Virginia Commonwealth University.

Doctors and parents say part of their frustration is that when they've asked the CDC about cases and deaths, they haven't received responses.

Rao says he reached out to a scientist on the CDC's AFM team, but never heard back.

"I would like answers," said Rao, an assistant professor of pediatric neurology at Children's Hospital of Richmond at Virginia Commonwealth University.

His voice chokes with emotion as he talks about Carter, whom he cared for for more than two years. "I want to know that the process benefits future cases of children with AFM," he said.

Other experts agree with Rao. "We want accurate numbers," said Dr. Kenneth Tyler, professor and chair of the department of neurology at the University of Colorado School of Medicine, and an adviser to the CDC on AFM.

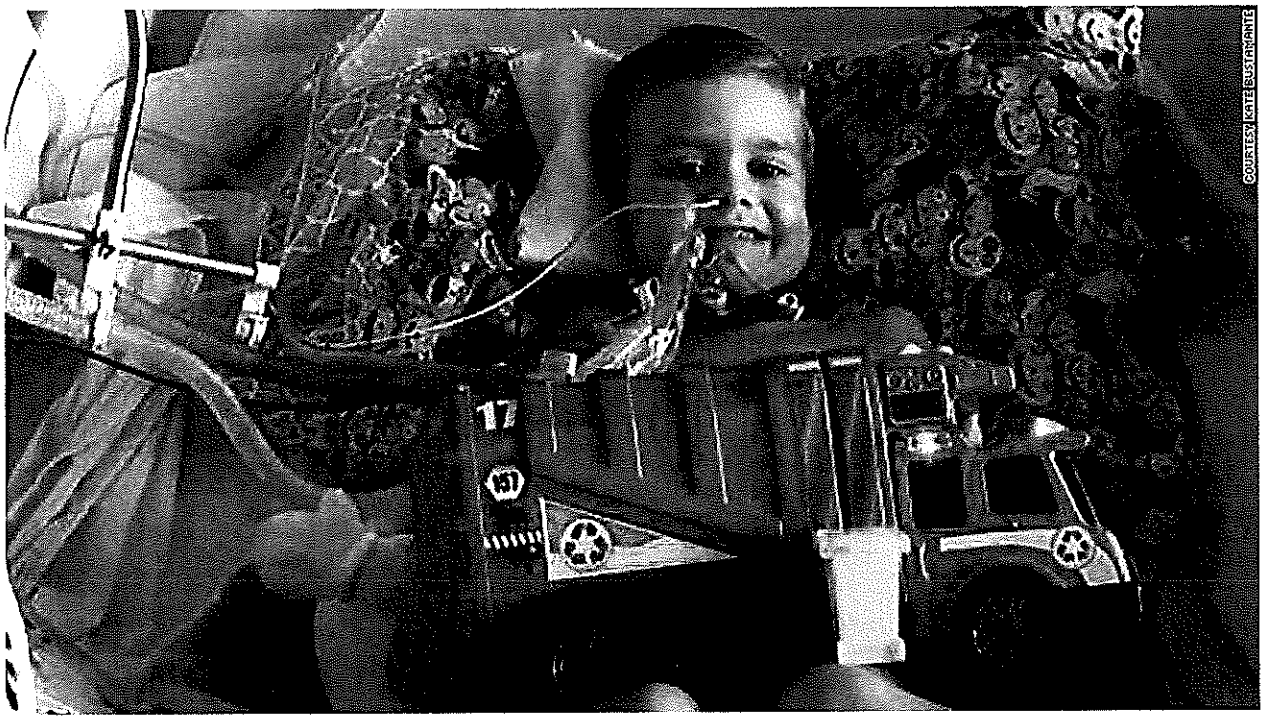
An accurate count could help answer questions, he said. Where are the cases? How old are the patients? Are they male or female? "That helps us understand causation," he said.

"The CDC is in the place of trying to understand the long-term [consequences], and a death is really important," added Van Haren, the pediatric neurologist at Stanford and adviser to the CDC.

Schuchat said the CDC is working hard on AFM. "We are working 24-7 on this and really take this seriously," she said.

She said the agency has established a case definition for AFM, done laboratory testing on samples sent to them by state health departments, and worked with outside experts to publish information in medical journals.

A CDC spokeswoman said in the past two weeks, the agency has added 14 officers from the Epidemiologic Intelligence Services -- known as "disease detectives" -- to help review AFM reports filed by state health departments.



In 2016, when Alex Bustamante was five years old, he had a headache, and then the next day developed paralysis. He became a paraplegic and a ventilator breathed for him. He died in May of this year.

An enterprising parent

On June 10, 2017, about ten months after Carter got sick, Robin reached out to the CDC.

She was angry.

"I know it may seem menial, a request from a clinically educated mother, but you are doing these children and family a disservice to not further educate physicians or demand surveillance similar to that of Zika," she wrote to an email address she found on the CDC's website.

"Please do what is right by public health standards and gather more data," she continued. "Or, god forbid if you know more than what you say in a limited fashion online please share it with the public and these patients. My son was immunized on schedule we observed very good hygiene precautions and still became a vent dependent quadriplegic overnight."

She received a response two days later.

"We are sorry to hear about your son," it said. "Your comments have been forwarded to the appropriate CDC program for their information. They will contact you directly if they have any questions."

She never heard from the CDC again.

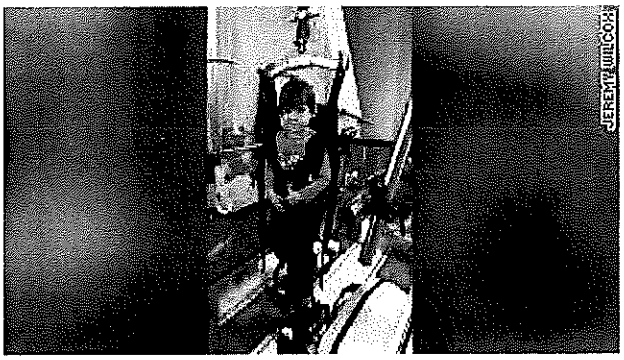
CNN relayed Robin's story to the CDC.

"I'm so sorry to hear that. That's very concerning," Schuchat told CNN. "We are trying to better connect with families, and it's so important that we listen to them."

Several other parents told CNN their emails to the CDC have also gone unanswered. Some of those emails offered help to the CDC from the families' Facebook group, which has gathered medical information on hundreds of AFM patients.

Messonnier, the CDC doctor, told CNN last month that she'd never heard of the Facebook group.

Last week another enterprising parent got a response from the federal agency. Four-year-old Joey Wilcox of Herndon, Virginia, was diagnosed with AFM in September. His father, Jeremy, works with government agencies as part of his job at a high-tech consulting firm. Wilcox knows how to seek out -- and find -- officials.



Joey Wilcox

He aimed high. Online he found Schuchat's email address, and asked her to meet with him and other parents. He says she responded within 15 minutes.

He thinks one of the reasons for the quick response is the "F" that parents gave the CDC.

That was a pivotal moment," he said of the CNN story.

More than a dozen families are scheduled to meet with Schuchat Tuesday in Washington.

Carter Roberts' and Alex Bustamante's parents will be among them.

CNN's John Bonifield and Michael Nedelman contributed to this story.

EXHIBIT NO.

17

Acute flaccid myelitis, the polio-like syndrome leaving some children partially paralyzed.NBC News
Oct. 25, 2018, 4:40 PM EDT
www.nbcnews.com/health/health-news/cdc-saya-polio-disease-puzzling-these-doctors-disagree-n924471
By Maggie Fox

The Centers for Disease Control and Prevention says it doesn't know what's causing a sudden rise in cases of a frightening, polio-like condition that leaves children paralyzed or with weakened limbs.

The No. 1 suspect had been a virus called enterovirus D68, or EV-D68. In 2014, a wave of cases of acute flaccid myelitis coincided with outbreaks of EV-D68 across the country.

But the CDC says it has not consistently found EV-D68 in confirmed cases since then. Officials say they're looking at a range of possible causes.

However, doctors who have been studying children affected by acute flaccid myelitis say they have gathered a growing body of evidence that EV-D68 is the main cause,

and that the virus may have changed in recent years in ways that make the paralyzing side-effects more likely.

They've documented an increase in cases of EV-D68 surrounding outbreaks of acute flaccid myelitis. Experiments have also shown that EV-D68 can invade nerve tissue, including the spine, and there's also evidence of genetic changes in the virus itself.

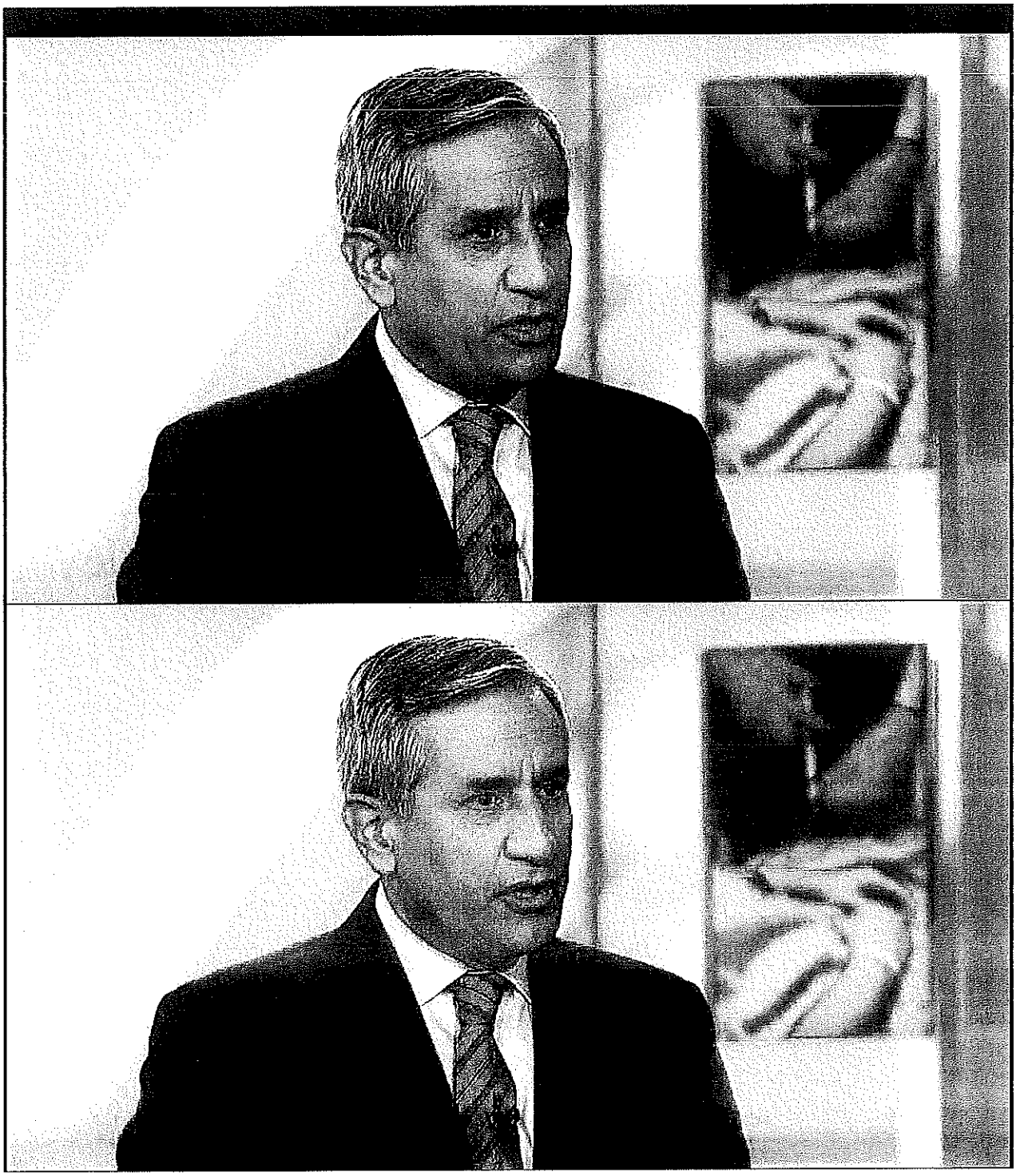
HEALTH

Doctors are struggling to find a cause of this paralyzing condition

A team of academic researchers have formed their own network to try to determine why a very few children develop the paralyzing syndrome from a virus that is harmless in more than 99 percent of those it infects.

"It's puzzling that four years later CDC has not confirmed the etiology of these cases," said Dr. Ali Khan, dean of the University of Nebraska Medical Center College of Public Health.

"Continuing to frame this as a mystery after so many years doesn't do the public health any justice," added Khan, a former director of CDC's Office of Public Health Preparedness and Response.



155 AFM cases under investigation, CDC confirms

State health departments have reported at least 191 possible cases of acute flaccid myelitis to the CDC this year, and the

agency says it has confirmed 72 of them. Other cases may also be related to viral infection, but they don't have the same precise symptoms of acute flaccid myelitis, which include sudden onset and specific damage to the spinal cord.

What's clear to the doctors who have treated many of the cases is that some virus is responsible, and that most cases are likely due to EV-D68.

"I think we are seeing the emergence of a new polio-like paralytic disease. Its pattern and most of the evidence that we have suggests that it is likely a virally caused disease," Dr. Ken Tyler, a neurologist at the University of Colorado School of Medicine, told NBC News.

HEALTH

The long road to recovery from acute flaccid myelitis. Some kids never recover.

"I think the leading candidate is enteroviruses in general and EV-D68 in particular."

Many different viruses circulate all at the same time, and one strain will be common one season and another strain the next.

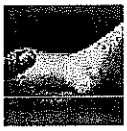
Increases in cases of acute flaccid myelitis were documented in 2014, 2016 and now in 2018. The CDC said there's not much evidence that EV-D68 cases also increased in 2016.

But Dr. Gregory Storch of Washington University in St. Louis and colleagues found an outbreak of EV-D68 in their region in 2016 that they said would have been missed if they had not actively looked for it in samples from children turning up in the St. Louis Children's Hospital with respiratory symptoms.

"It does seem that enterovirus D68 has been present every other year," Storch said. "Each of those times, there has been an increase in acute flaccid myelitis that occurred at the same time as the EV-D68 activity."

Dr. Kevin Messacar of Children's Hospital Colorado and colleagues similarly found an increase in EV-D68 cases in Colorado in 2016.

And Guiqing Wang, a pathologist at New York Medical College, and colleagues did genetic testing to identify a new strain of EV-D68 that had circulated in both 2014 and 2016. It caused an outbreak of 160 confirmed cases in the Lower Hudson Valley of New York in 2016, they reported.



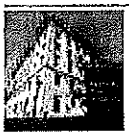
HEALTH

Hand, foot and mouth disease has college students hiding in dorms

"It looks like the new strains are better able to affect human motor neuron-like cells than older strains," said Tyler.

It wasn't just the U.S.: European researchers reported 29 cases of EV-D68 infection in children and adults with acute flaccid myelitis in 2016.

Illinois refuses permit for concert to be hosted by R. Kelly



Shutdown blocks help for identity theft victims, as FTC goes dark

Tyler and colleagues infected mice with EV-D68 and showed it could affect the nervous system. "We know that enterovirus D68 in mice can produce an illness very much like what you see in children," Tyler said. "You can see enterovirus particles in the motor neurons."

It's not proof, Tyler said. "It means you have established that it is plausible that a virus does behave in this way," he said.

What's missing is the surveillance needed to show that outbreaks of EV-D68 were large enough to account for the increases in cases of AFM in 2016 and now in 2018, and that most patients with AFM were also infected with EV-D68.

TEST QUICKLY TO CATCH THE VIRUS

There's also a need to find out what other viruses might be causing cases. Messacar noted that a virus called EV-A71 caused outbreaks of hand, foot and mouth disease this fall in Colorado, and that virus has also been linked to neurological effects.

HEALTH

What is acute flaccid myelitis?

That means doctors need to act more quickly to test kids who begin showing signs of weakness or paralysis. That might not be the first priority if a child comes to the emergency room with trouble breathing or an arm that suddenly doesn't work.

But a virus can cause damage to the nervous system and then disappear when the immune response kicks in — leaving a crime scene with little evidence of the perpetrator. That adds to the mystery.

"Other data from 2014 suggest that the faster you did the swabs, the higher the yield was," Tyler said. After a week or so, it was hard to find any virus in the patients, he said.

"One of the things we need to do more of going forward is active surveillance," Messacar said.

It's also possible that the damage is not directly caused by the virus, but by an abnormal immune response to the infection. That makes it even more important to catch the virus in the act.

A few days can make all the difference, said Dr. Aaron Milstone, a pediatrician at Johns Hopkins Medicine who is part of an informal network of researchers trying to get to the bottom of what has caused the increase in AFM cases.

19 Benefits All Military Personnel & Veterans Didn't Know They Could Get Veterans Discounts Club

"This is a scary phenomenon that we are seeing, but it is uncommon. It is very unlikely that one's child will develop this."

They're telling doctors to get tests as quickly as possible from patients, including respiratory swabs, stool samples and spinal taps. "These are common areas where we would find enteroviruses," Milstone said.

"As soon as you have that presentation of weakness or paralysis, that's when you should get that testing done — immediately. Don't wait a few days."

Milstone says his network is also trying to figure out what it is about the patients that makes them susceptible to paralysis, possibly some genetic predisposition.

"We have been recruiting patients for four years now for a genetics study and it is really hard when patients are all over the country," he said.

"We have sent emails to institutions across the county and say, 'hey, do you know of anyone?'"

None of this means parents need to worry if their child develops a cold. "We don't want people rushing to the pediatrician just because their kid has a stuffy nose," Milstone said.

"This is a scary phenomenon that we are seeing, but it is uncommon. It is very unlikely that one's child will develop this."

Maggie Fox

Maggie Fox is a senior writer for NBC News and TODAY, covering health policy, science, medical treatments and disease.

EXHIBIT NO.

18

More US kids get paralyzing illness, cause is still unknown

AP
MIKE STOBBE

Associated Press•November 13, 2018

NEW YORK (AP) — More children have been diagnosed with a mysterious paralyzing illness in recent weeks, and U.S. health officials said Tuesday that they still aren't sure what's causing it.

This year's count could surpass the numbers seen in similar outbreaks in 2014 and 2016, officials said. Fortunately, the disease remains rare: This year, there have been 90 cases spread among 27 states, the Centers for Disease Control and Prevention said.

It's not clear what's causing some children to lose the ability to move their face, neck, back, arms or legs. The symptoms tend to occur about a week after the children had a fever and respiratory illness.

Health officials call the condition acute flaccid myelitis. No one has died from it this year, but CDC officials say at least half the patients do not recover from the paralysis and some have serious complications.

Polio and West Nile virus have been ruled out. Doctors have suspected the cause might be some kind of enterovirus, which in most people causes cold symptoms. But CDC officials say that's not clear.

The first mysterious wave of paralysis cases in 2014 coincided with a wider spike in illnesses connected to an enterovirus called EV-D68, CDC officials said. But there was no such spike when another wave occurred in 2016, or this year.

There's also a lack of clinical evidence: CDC officials have checked the spinal fluid of about three-quarters of the 90 patients, and found EV-68 in only one. Another type of enterovirus called EV-A71 was found in only one other patient.

But there are questions about that, too. If a virus is the cause, it's possible the test is not good enough, or the germ cleared the spinal fluid by the time the tests were taken, or the culprit is hiding elsewhere in the body, said the CDC's Dr. Nancy Messonnier.

It's also possible the paralyzing illnesses are caused by some new germ for which no lab test has been developed. Or maybe there's some predisposing factor in some patients that cause their immune systems to react so severely to a germ or other trigger that the immune response causes paralysis, CDC officials said.

Parents and even some scientists have criticized the agency for not solving the riddle.

"I understand why parents are frustrated. I'm frustrated. I want answers too," said Messonnier, who is overseeing the agency's outbreak investigation. CDC officials have pledged to do more to notify doctors to look for possible cases and to more thoroughly review cases from years past for further clues.

About 120 cases were confirmed in 2014, the first time such a wave occurred. Another 149 were reported in 2016. In 2015 and 2017, the counts were far lower, and it's not clear why.

The illnesses have spiked in September each year there's been a wave and then tailed off significantly by November. But it can take weeks to determine if they should be counted in the outbreak. More than 160 cases are being investigated, and some of those may join the count, CDC officials said.

The Associated Press Health & Science Department receives support from the Howard Hughes Medical Institute's Department of Science Education. The AP is solely responsible for all content.

EXHIBIT NO.

19

www.eurosurveillance.org/content/10.2807/1560-7917.ES.2018.23.3.17-00310 (18 Jan 2018)

The association between acute flaccid myelitis (AFM) and Enterovirus D68 (EV-D68) – what is the evidence for causation?

- Amalie Dyda[1], Sacha Stelzer-Braid[2,3], Dillon Adam[1], Abrar A Chughtai[1], C Raina MacIntyre[1,4]
- View Affiliations Hide Affiliations

Affiliations: [1]School of Public Health and Community Medicine, University of New South Wales (UNSW), Sydney, New South Wales (NSW), Australia[2]School of Medical Sciences, University of New South Wales (UNSW), Sydney, New South Wales (NSW), Australia[3]Division of Serology and Virology, South Eastern Area Laboratory Services, Prince of Wales Hospital, Sydney, Australia[4]College of Public Service and Community Solutions and College of Health Solutions, Arizona State University, Tempe, Arizona, United States

Correspondence:

Dillon Adam

- View Citation Hide Citation

Citation style for this article:Dyda Amalie, Stelzer-Braid Sacha, Adam Dillon, Chughtai Abrar A, MacIntyre C Raina. The association between acute flaccid myelitis (AFM) and Enterovirus D68 (EV-D68) – what is the evidence for causation?. Euro Surveill. 2018;23(3):pii=17-00310. https://doi.org/10.2807/1560-7917.ES.2018.23.3.17-00310Received: 04 May 2017; Accepted: 11 Oct 2017

Background

Enterovirus D68 (EV-D68) has historically been a sporadic disease, causing occasional small outbreaks of generally mild infection. In recent years, there has been evidence of an increase in EV-D68 infections globally. Large outbreaks of EV-D68, with thousands of cases, occurred in the United States, Canada and Europe in 2014. The outbreaks were associated temporally and geographically with an increase in clusters of acute

flaccid myelitis (AFM). **Aims:** We aimed to evaluate a causal association between EV-D68 and AFM. **Methods:** Using data from the published and grey literature, we applied the Bradford Hill criteria, a set of nine principles applied to examine causality, to evaluate the relationship between EV-D68 and AFM. Based on available evidence, we defined the Bradford Hill Criteria as being not met, or met minimally, partially or fully. **Results:** Available evidence applied to EV-D68 and AFM showed that six of the Bradford Hill criteria were fully met and two were partially met. The criterion of biological gradient was minimally met. The incidence of EV-D68 infections is increasing worldwide. Phylogenetic epidemiology showed diversification from the original Fermon and Rhyne strains since the year 2000, with evolution of a genetically distinct outbreak strain, clade B1. Clade B1, but not older strains, is associated with AFM and is neuropathic in animal models. **Conclusion:** While more research is needed on dose–response relationship, application of the Bradford Hill criteria supported a causal relationship between EV-D68 and AFM.

Article metrics loading...

- Click here for cited by

- From This Site
- PubMed
  - PubMed citation
  - Amalie Dyda
  - Sacha Stelzer-Braid
  - Dillon Adam
- Google Scholar
  - Amalie Dyda
  - Sacha Stelzer-Braid
  - Dillon Adam
  - Search for related Content

- ISSN: 1025-496X, Online ISSN: 1560-7917
- DOI: http://dx.doi.org/10.2807/1560-7917.ES.2018.23.3.17-00310
- Volume 23, Issue 3

Go to section...

Amalie Dyda[1]; Sacha Stelzer-Braid[2,3]; Dillon Adam[1]; Abrar A Chughtai[1]; C Raina MacIntyre[1,4]

1: [1]School of Public Health and Community Medicine, University of New South Wales (UNSW), Sydney, New South Wales (NSW), Australia; **2:** [2]School of Medical Sciences, University of New South Wales (UNSW), Sydney, New South Wales (NSW), Australia; **3:** [3]Division of Serology and Virology, South Eastern Area Laboratory Services, Prince of Wales Hospital, Sydney, Australia; **4:** [4]College of Public Service and Community Solutions and College of Health Solutions, Arizona State University, Tempe, Arizona, United States

ABSTRACT

Go to section...

- **Background:**

  Enterovirus D68 (EV-D68) has historically been a sporadic disease, causing occasional small outbreaks of generally mild infection. In recent years, there has been evidence of an increase in EV-D68 infections globally. Large outbreaks of EV-D68, with thousands of cases, occurred in the United States, Canada and Europe in 2014. The outbreaks were associated temporally and geographically with an increase in clusters of acute flaccid myelitis (AFM).**Aims:** We aimed to evaluate a causal association between EV-D68 and AFM. **Methods:** Using data from the published and grey literature, we applied the Bradford Hill criteria, a set of nine principles applied to examine causality, to evaluate the relationship between EV-D68 and AFM. Based on available evidence, we defined the Bradford Hill Criteria as being not met, or met minimally, partially or fully. **Results:** Available evidence applied to EV-D68 and AFM showed that six of the Bradford Hill criteria were fully met and two were partially met. The criterion of biological gradient was minimally met. The incidence of EV-D68 infections is increasing world-wide. Phylogenetic epidemiology showed diversification from the original Fermon and Rhyne strains since the year 2000, with evolution of a genetically distinct outbreak strain, clade B1. Clade B1, but not older strains, is associated with AFM and is neuropathic in animal models. **Conclusion:** While more research is needed on dose–response relationship, application of the Bradford Hill criteria supported a causal relationship between EV-D68 and AFM.

Keywords:

- Enterovirus D68,
- acute flaccid myelitis,

- acute flaccid paralysis,
- Bradford Hill Criteria,
- AFM,
- EV-D68

Background

Go to section...

- Large outbreaks of enterovirus D68 (EV-D68), affecting at least 2,287 people, occurred in multiple countries in 2014. The first outbreak was detected in the United States (US), followed by Canada, Europe and Asia. At the same time, an increase in clusters of acute flaccid myelitis (AFM) occurred in the same geographical areas, with the highest number of cases (n = 120) in the US [1-3]. Smaller numbers of AFM cases (at least six) associated with EV-D68 infection were reported from Denmark, France, the Netherlands, Spain, Sweden and the United Kingdom (UK) [1-3]. This raised the question of causal association between EV-D68 and AFM [1-3].

The enterovirus (EV) genus comprises 12 distinct species [4], including poliovirus. EV-D68 belongs, with other non-polio EVs, to the group EV D [5,6]. AFM is defined as 'acute flaccid paralysis in one or more limbs or acute onset of bulbar paralysis' and if caused by poliovirus, is referred to as poliomyelitis or polio [7]. In addition to poliovirus as the leading cause of AFM [8,9], other EVs such as EV-A71 are also a recognised cause of AFM [10,11]. Following the 2014 epidemic, EV-D68 has emerged as another possible cause of AFM [12].

Infection with EV-D68 historically caused mild respiratory symptoms such as rhinorrhoea, muscle aches and cough. Recently however, it has been associated with severe respiratory symptoms, hospitalisation and death [13]. Children are at higher risk of symptomatic infection than adults [2]. Transmission occurs from person to person, and the virus is found in respiratory secretions, blood and infrequently in cerebrospinal fluid (CSF) [13,14].

EV-D68 was first identified in 1962 and has since been found sporadically and in small clusters, with only 699 confirmed cases worldwide until 2013 [15,16]. From 2008 to 2010, the US Centers for Disease Control and Prevention (CDC) reported the identification of six clusters of respiratory illness associated with EV-D68 in Asia, Europe and the US, with the number of confirmed cases ranging from five to 28 [2]. From 2009 to 2013, a total of 79 cases were reported in the US, with generally mild symptoms [1], followed by 1,153 cases in 2014 [13]. In addition, an outbreak involving 25 people occurred in June 2016 in the Netherlands [17].

The global prevalence of EV-D68 as a cause of illness appears to be low. Studies have reported the proportion of respiratory specimens positive for EV-D68 ranging from 0.2 to 3.4% [18-21]. A study conducted from 2011 to 2015 in China found that 12 of 7,945 (0.2%) specimens were positive for EV-D68 [21]. In Hong Kong an investigation in children showed that 24 of 1,461 (1.6%) of respiratory samples were positive for EV-D68 [19]. In Germany, where respiratory samples were collected from patients in three hospitals between January 2013 and December 2014 [18], 39 of 14,838 (0.3%) were positive for EV-D68 [18]. Data from the French enterovirus surveillance network showed that from July to

December 2014, 212 of 6,229 (3.4%) samples were positive for EV-D68 [20]. However, prevalence may be affected by geographical location, type of test conducted and testing practices (such as changes in likelihood of testing for EV-D68 during an epidemic, owing to increased awareness among clinicians of the disease and the availability of tests).

The first detected large-scale outbreak of EV-D68 was reported in the US and Canada in 2014 [3,22,23] and was associated with severe respiratory symptoms [13]. The outbreak occurred from August 2014 to January 2015 (autumn/winter), with a total of 1,153 cases in 49 US states [13]. The disease severity was much higher than in previous outbreaks, with higher numbers of people experiencing severe respiratory disease. AFM was diagnosed in 10.4% (120/1,153) of cases in the US and five cases of neurological illness were associated with EV-D68 in Canada [11,22,24,25].

Outbreaks were also reported in Europe. Investigation into an increase in the number of children with severe respiratory disease in September 2014 in a hospital in Norway found that 33 of 303 (10.9%) paediatric samples were positive for EV-D68 [26]. The hospital reported two cases of severe AFM associated with EV-D68 infection [27]. A sporadic case of AFM following EV-D68 infection was also reported in France [28] and the UK in 2014 [29].

Phylogenetic analysis of a section of the EV-D68 genome (the VP1 gene) has identified four distinct clades of EV-D68, named A, B, C and D [15,30]. Clade B can be further divided into B1, B2 and B3 [31]. Strains isolated in the US from the 2014 outbreak belonged mostly to clade B [30], in particular B1 [12].

Despite the growing concern following the 2014 outbreak and the association with AFM in several countries, information regarding causation between EV-D68 and AFM is still limited. We aimed to evaluate evidence of a causal relationship between EV-D68 and AFM using the Bradford Hill criteria.

Methods

Go to section...

A review of the literature was conducted in April 2017 using MEDLINE and EMBASE. Systematic search terms used were 'enterovirus 68' OR 'EV-D68' or 'EV68' AND 'acute flaccid myelitis' OR 'acute flaccid paralysis'. The search was restricted from 1980 to present. A search of the grey literature using Google was also conducted using the same search terms. All bibliographies of included studies were reviewed. Studies were included if they investigated an association between AFM and EVs, described a case series of EV-D68 and AFM or investigated the biology of EV-D68. Studies were excluded if they investigated EVs in general, i.e. not specifically EV-D68 or an association with AFM. The Bradford Hill criteria were applied to the evidence.

Bradford Hill criteria

The Bradford Hill criteria are a set of nine criteria applied to examine causality between an exposure and a disease (Table 1) [32].

Table 1

Bradford Hill criteria of causality

Toggle display:Table 1 Open Table 1 fullscreen

Criterion	Description
Strength	Whether those with the exposure are at a higher risk of developing disease and if so, how much more risk? This criterion suggests that a larger association increases the likelihood of causality.
Consistency	The credibility of findings increases with repetition of findings, including consistency of study findings across different populations and geographical locations.
Specificity	Causality is more likely if the exposure causes only one specific disease or syndrome, or if a specific location or population are being affected.
Temporality	This criterion requires that the exposure must occur before the disease, and not after a latency period that is too long. This criterion must always be fulfilled for causality to be concluded.
Biological gradient	The argument for causality is stronger in the presence of a dose–response relationship, where higher or longer exposure leads to an increased risk of disease.
Plausibility	A conceivable mechanism for causation between disease and exposure should exist for there to be a causal relationship.
Coherence	The current association should not contradict any previous knowledge available about the disease and/or exposure.
Experiment	This criterion can involve scientific experiments and addresses the association of exposure with disease. However, 'experiment' relates to the decrease in disease risk when the exposure is removed and often involves animal models.
Analogy	This criterion uses previous evidence of an association between a similar exposure and disease outcome to strengthen the current argument for causation.

Source: [32].

Application of the criteria for EV-D68 and AFM

The literature was reviewed to gather evidence relevant to each of the Bradford Hill criteria. Key findings from each identified study were assessed against the relevant criteria. Each criterion was qualitatively assigned one of four categories based on the amount of information available, sample size in the studies and the certainty of the findings:

The fit of the evidence to the criteria was scored as follows (Table 2):

Table 2

Qualitative evaluation of the Bradford Hill criteria

Toggle display:Table 2 Open Table 2 fullscreen

Fulfilment of each Bradford Hill criterion	Qualitative score
The criterion is fully met	+ + +
The criterion is partially met	+ +
The criterion is minimally met, with some aspects being consistent	+
The criterion is not met	–

Given the possible subjectivity in assigning scores, we present a summary of available evidence and justification to support the score for each criterion.

Results

Go to section...

The results from the literature review are described below in the Figure.

Figure

Study selection, literature review on enterovirus D68 and acute flaccid paralysis (n = 123)

...

The evaluation of the available evidence against the Bradford Hill criteria is summarised in Table 3 and described in more detail below to justify the final scoring.

Table 3

Rating of the evidence for causation between EV-D68 infection and acute flaccid myelitis

Table 3

Rating of the evidence for causation between EV-D68 infection and acute flaccid myelitis

Toggle display:Table 3 Open Table 3 fullscreen

Criterion	Qualitative score
Strength	+ +
Consistency	+ + +
Specificity	+ +
Temporality	+ + +
Biological gradient	+
Plausibility	+ + +
Coherence	+ + +
Experiment	+ + +
Analogy	+ + +

Strength

As at April 2017, there have been two epidemiological studies investigating the causation between EV-D68 infection and cases of AFM. Both were retrospective case–control studies following the 2014 outbreak in the US. The first was conducted in Colorado from 3 August to 18 October 2014. The study compared AFM cases with two control groups. Eleven cases of AFM were identified, of whom four tested positive for EV-D68. Multivariate analysis showed that those with AFM had higher odds of EV-D68 infection than both control groups (adjusted odds ratio (OR) = 10.3; 95% confidence interval (CI): 1.8–64.8 and OR = 4.5; 95% CI: 1.0–21.2) [33]. The second study was conducted in Colorado and California in patients with AFM between January 2012 and October 2014 as well as cases identified from a state-wide surveillance system in the same period [12]. EV-D68 was detected in nasopharyngeal or oropharyngeal samples in 12 of 25 patients with AFM. Among the 11 cases with AFM from two linked clusters during the 2014 outbreak, four tested positive for EV-D68. None of the total 25 AFM patients tested positive for the virus in cerebrospinal fluid (CSF). However, the association was strengthened as no other infections were identified in CSF to explain the AFM. The findings from this study are limited by the small sample size.

A summary of these two studies is shown in Table 4 [12,33]. Given that only two studies calculated odds ratios for the association, even though convincing, we judged, based on the small number of available studies, that the criterion of strength was partially met.

Table 4

Summary of epidemiological studies investigating strength or consistency of association

Toggle display:Table 4 Open Table 4 fullscreen

Criterion	Study	Study period	Number of AFM cases	Proportion with EV-D68
Strength	Aliabadi et al. [33]	3 August–18 October 2014	11	4/11
	Greninger et al. [12]	2012–2014	25	12/25
Consistency	Ayscue et al. [38]	2012–2014	23	2/19
	Pastula et al. [35]	2014	9	4/9
	Messacar et al. [36]	2014	12	5/11
	Sejvar et al. [25]	2014	120	11/56
	Van Haren et al. [37]	2012–2015	59	9/45

AFM: acute flaccid myelitis; EV-D68: enterovirus D68.

Consistency

The association between EV-D68 and AFM is generally consistent, with reports across at least nine countries (Canada, Denmark, France, the Netherlands, Norway, Spain, Sweden, the UK and the US) and different outbreaks. An increase in cases of both EV-D68 and AFM was observed in the 2014 outbreak across the US [11] and Canada [12]. A total of 120 cases of AFM were reported during this time [24,25,34]. A cluster of nine acute neurological illness cases was initially identified in Colorado in September 2014, of whom four were found to be positive for EV-D68 [35]. A second study looked at this cluster and identified 12 children with AFM, of whom 11 were tested for EV-D68; five of those 11 were positive for EV-D68 [36]. However, the strength of the above associations cannot be quantified without control data. Reports from CDC in November 2014 identified 88 cases of AFM; among the 88 cases of AFM, 41 upper respiratory tract specimens were tested, eight of which were positive for EV-D68. Of the 19 samples taken less than 14 days following symptom onset, seven tested positive for EV-D68 [37]. US national surveillance following the 2014 outbreak found 120 cases of AFM, with one CSF specimen positive and 11 of 56 upper respiratory specimens positive for EV-D68 [25]. A summary of these outbreaks is described in Table 4.

In addition, between 2012 and 2014, 23 cases of AFP were identified in California of whom two tested positive for EV-D68 [38]. Other cases of AFM have been identified in people infected with EV-D68: two in Norway [27], one in France [28] and one in the UK [29]. A study in Western Australia investigating the epidemiology of EV-D68 found three cases

who developed AFM [39]. In addition, one case of AFM was identified in a cluster of 25 EV-D68 cases in the Netherlands between June and July 2016 [17].

There have also been a number of case series describing AFM and EV-D68. In a retrospective US-wide study investigating patients diagnosed with AFM during the 2014 outbreak, 11 of 56 respiratory specimens were EV-D68-positive. One of 55 CSF specimens was positive for EV-D68, however all stool/rectal swabs (n = 54) were negative. Eight of 17 cases tested within less than 7 days of symptom onset were positive for EV-D68. The low proportion of positive specimens could also be related to a delay of days between symptom onset and sampling [25]. A case series investigating AFM in California (June 2012 and July 2015) found 9 of 45 AFM cases cases infected with EV-D68 [37]. A second case series in California, from 2012 and 2014, identified 23 cases of AFP of whom two tested positive for EV-D68 [38]. A cluster of three cases of AFM were identified in 2014 in Alberta, Canada, of whom two tested positive for EV-D68 [40]. However, while these descriptive data are suggestive of a causal association, they are limited by being a small case series.

The available evidence from multiple studies showed consistency and suggested that this criterion was met.

Specificity

Specificity in The Bradford Hill criteria can refer either to one specific exposure causing one disease or more broadly to the argument for causation being strengthened if a disease is affecting one specific population group with a similar exposure. AFM can be caused by a range of exposures and is therefore not specific to EV-D68. However, the simultaneous occurrence of EV-D68 and AFM does appear to affect a specific population, namely children: the majority of participants in a retrospective cohort study of patients of any age in the US were children diagnosed with AFM [12], while a cluster of nine cases identified in Colorado in September 2014 consisted only of people aged less than 18 years [35]. In a cluster of three cases reported in Alberta, Canada, all cases were in children aged from 5 to 15 years who had underlying respiratory conditions [40]. Other reports suggest an association between AFM and non-polio EVs which specifically affects immunocompetent children in North America [35,38].

The available evidence suggested that the criterion of specificity was partially met.

Temporality

There is a strong temporal relationship between EV-D68 and AFM. In the 2014 US outbreak the number of EV-D68 cases increased at the same time as polio-like illness in children. In addition, the outbreak of EV-D68 subsided around the same time as the cases of AFM, with cases of both decreasing in October 2014 [25]. As mentioned previously, Sejvar et al. detected EV-D68 in eight of 17 respiratory specimens from patients with AFM collected no more than 7 days from onset of limb weakness [25].

The available evidence suggested a clear temporal relationship, with AFM following outbreaks of EV-D68 in time; the criterion of temporality was met.

Biological gradient

There is some evidence for biological gradient. This was shown in suckling mice which were inoculated with four different strains of EV-D68 and then observed for a 2-week period [41]. The muscle and brain tissues of the mice were harvested and passaged to new mice. Limb tremors and weakness were observed after the Rhyne strain of the virus was passaged twice. Once the virus was passaged three times, the mice infected with the passaged strain developed paralysis and died [41].

There has been some investigation into animal models for EV-A71 infection using non-human primates: neurological symptoms in cynomolgus monkeys with EV-A71 infection were similar to those seen in humans [42]. Infection of EV-A71 in mouse models proved ineffective, owing to an incompatible murine scavenger receptor class B2 (SCARB2) receptor protein used for virus binding in humans. To circumvent this issue, Victorio et al. [43] developed a mouse-adapted EV-A71 strain. This strain induced clinical signs including paralysis and acute encephalomyelitis in 1-week-old BALB/c mice. Although this has helped increase the understanding of EV-A71 infection, an ideal animal model that can be infected with clinical EV-A71 strain has not yet been identified [44].

This criterion could be investigated further in animal models, using information on viral load where available, comparing EV-D68 viral load in patients with AFM to viral load in patients with mild EV-D68 infection. A study in 2016 concluded that, similar to rhinovirus infections, higher EV-D68 viral load may indicate more symptomatic disease [17]. Studies on viral load of EV other than EV-D68 in CSF detected an average of 10,000 to 100,000 copies/mL in adults and children [45,46]. Higher viral load was associated with increased vertigo and paraesthesia and increased leukocytes and proteins in the blood but not with fever and headache [46]. Certain EV genotypes including E30 and Coxsackie virus B (CVB 4 and 5) were also associated with increased viral loads [46]. A mouse model showed that multiple clades of EV-D68 were neurovirulent and caused paralysis, but the study did not specifically examine the dose–response relationship [47].

The available evidence from studies of other EVs and some data on EV-D68 was suggestive of a biological gradient, but because studies designed to address specifically a dose–response relationship were lacking the criterion of biological gradient was minimally met.

Plausibility

It is biologically plausible that EV-D68 can lead to AFM. EVs are associated with neurological complications, most notably EV-A71, which has been linked to severe neurological complications including AFM and encephalomyelitis during hand foot and mouth disease (HFMD) outbreaks [48]. While EV-A71 has been shown to utilise many different receptors for facilitated cell entry, the scavenger receptor class B2 (SCARB2) protein has been most widely implicated in facilitating EV-A71 neuropathies [49]. SCARB2 is widely expressed throughout the human body, including on neurons of the CNS [50]. Furthermore, EV-A71 inoculation in a SCARB2-transgenic mouse induced encephalomyelitis clinically analogous to that observed in humans [51]. In contrast, CV-A16, which can similarly bind to SCARB2 for entry into cells, is not a neurotropic virus [49].

This suggests either that EV-A71 uses an alternative receptor for neurovirulence or that other unknown factors may inhibit entry of CV-A16, but not EV-A71, into neurons [50].

While it is known that D68 binds to cells in the respiratory tract via sialic acid receptors, the molecular basis its neurotropic pathway has yet to be established [52,53]. Like for EV-A71, the neurotropic pathway and subsequent neuropathies of EV-D68 may be an infrequent diversion from the normal replication cycle in the respiratory mucosa. There is evidence that EV-D68 is capable of infecting nerve cells, with one study showing virus detected in the CSF of a young adult with AFM in 2005 [14]. In 2008, EV-D68 was detected during autopsy in the brain and CSF of a 5-year-old boy with fulminant encephalitis [54]. In a 2015 study, the virus was detected in CSF from a 25 year-old man [39]. A retrospective study of the 2014 outbreak in the US also found a CSF specimen positive for EV-D68 in a patient with AFM [25]. However, despite these examples, most cases of AFM associated with EV-D68 have been negative for the virus in CSF. Both poliovirus and EV-A71 are rarely detected in the CSF, even among cases with AFM [55,56]. Poliomyelitis is typically confirmed by detection of virus in the stool, while EV-A71 is usually detected in the respiratory tract [25]. For this reason, the lack of EV-D68 in the CSF of AFM cases does not weaken plausibility. A mouse study showed that EV-D68 has tropism for spinal cord motor neurons and does not replicate efficiently in brain or other tissues [47]. The clinical syndrome in mice with EV-D68-induced paralysis shows a lower motor neuron pattern, with the upper limbs more affected and, corresponding to pathological findings, no cerebral or sensory involvement. This is consistent with AFM and supports EV-D68 causing AFM in humans.

The available evidence on EV-D68 and published knowledge about EVs in general, suggested that the criterion of plausibility was met.

Coherence

The criterion for coherence was met because the current hypothesis that there is an association between EV-D68 and AFM did not contradict any prior knowledge about EV-D68. EVs have been shown to affect the nervous system and can cause other neurological complications [10,57,58]. Specifically, EV-A71 has been associated with severe neurological complications including AFM and encephalomyelitis during outbreaks of HFMD [48]. The available evidence suggested that this criterion was met.

Experiment

There is some experimental evidence regarding the pathogenesis of EV-D68, but this is not specific to AFM. As mentioned in the section on biological gradient above, an experiment in mice showed that one strain produced weakness, limb tremors, paralysis and death, demonstrating neurovirulence of EV-D68 [41].

Recent studies showed genetic differences between EV-D68 strains from China, where outbreaks with milder disease occurred in 2015 and 2016, and the EV-D68 strains from the more severe US 2014 outbreak [59]. In addition, genetic analysis of EV-D68 strains from the US 2014 outbreak demonstrated that most strains belonged to clade B which is

associated with severe disease and had not been detected in the US before the outbreak [30]. Analysis of EV-D68 strains globally indicated increasing genetic diversity and the evolution of clade B1 since 2000 [47].

A mouse study published in 2017 showed that strains of EV-D68 from the 2014 epidemic induced AFM in mice similar to the human clinical syndrome, with virus detected in the motor neurons [47]. The authors also showed that phylogenetically older strains (such as the Fermon strain) did not cause AFM. While only clade B1 has been isolated from human AFM patients [12], an experimental mouse study [47] identified neurovirulent strains from multiple clades (A, B, and B1). The study further demonstrated that Koch's postulates were fulfilled when infecting naïve mice with strains from affected mice. Antibodies from infected mice protected naïve mice. The study also showed that viral RNA was detectable in tissue and CSF longer than infectious viral particles which do not grow well or persist for long in cerebral tissue and CSF. Intramuscular introduction of the virus resulted in 100% of the mice becoming paralysed, in contrast to much lower rates following intranasal or intraperitoneal inoculation.

The available evidence suggested that the experiment criterion was met.

Analogy

This criterion was met in this investigation of causation, as there is suggestive evidence of AFM being associated with other EVs including EV-A71 and EV-D70 [10]. EV-A71 has been reported as one of the most important EVs capable of infecting nerve cells and has caused numerous outbreaks of paralytic disease [60,61]. In the Asia-Pacific region, a number of outbreaks of HFMD with associated complications such as AFM have been reported. A descriptive study was conducted following one such outbreak in Taiwan in 1998 in which more than 55 children died. Of 41 patients with neurological complications and EV-A71 infection, four had AFM. EV-A71 has also been shown to lead to complications such as aseptic meningitis, brain-stem encephalitis and rhombencephalitis [10].

The available evidence suggested that the criterion of analogy was met.

Discussion

Go to section...

Historically, the incidence of EV-D68 has been low, with sporadic cases and small clusters of mild illness reported. Whether this represents under-ascertainment or true low incidence in the past is unclear, but active EV surveillance studies in several countries including Germany in 2013-14, Hong Kong in 2014, France in 2014 and China in 2011-15 suggest that EV-D68 was a rare cause of clinical infection in the past [18-21]. Since 2014, the number of reported infections and clusters has increased. In addition, severe complications including AFM have been reported since 2014 [3]. Several clusters of AFM in recent years were associated with EV-D68 and a large outbreak of EV-D68 in 2014 in the US was associated with severe respiratory illness.

Our application of the Bradford Hill criteria suggested good evidence for EV-D68 being a cause of AFM. While EVs in general are neurotropic, AFM has never previously been associated with EV-D68. It could be that incidence of EV-D68 was genuinely been much lower in the past, so that rare complications of infection have not been apparent. An analogous example is the association between Zika virus and microcephaly which was only recognised during a large-scale epidemic in Brazil in 2015 [62,63]. However, retrospective analysis of a large outbreak of Zika virus in French Polynesia two years earlier showed the same association with microcephaly but was not recognised at the time [64]. In addition, clades A, B and B1 of EV-D68 were highly neurovirulent in animal studies, with specific tropism for motor neurons [47]. It appears that these strains which evolved after the year 2000 are capable of causing AFM, as demonstrated in a mouse model, while the original Fermon and Rhyne strains do not cause AFM [47].

There is a need for phylogeographic epidemiology to ascertain temporal and geographic changes in the virus and whether such changes could explain why AFM is newly associated with the virus. Genetic changes in the virus which have rendered it more neuropathic could explain the association, and several clades have been shown to be highly neurovirulent. Phylogenetic studies have demonstrated that strains isolated in recent outbreaks are very divergent from the original Fermon strain isolated in 1962 [15]. Clades B1 and B2 caused the 2014 outbreak [30] and clade B3 caused an outbreak of severe EV-D68 infection in the Netherlands in 2016 [17]. Strains in clade B1 have mutations in structural and non-structural proteins, which could play a role in the reported neurovirulence of these strains [12], and all EV-D68-infections in human AFM cases were attributed to clade B. However, mouse studies showed that multiple clades (A, B and B1) cause paralysis [47]. The observation that clade B1 was associated with AFM in 2014 may be due to the much higher incidence of clade B1 infection in 2014. More research is needed to study biological gradient and to quantify measures of association between EV-D68 and AFM.

Given that the association of AFM with EV-D68 is recent, there is a strong case for systematic and enhanced EV surveillance, which will enable investigation of epidemiological data for measures of association. While past studies and EV surveillance showed that EV-D68 was a rare cause of EV infection, there has been a change in disease epidemiology since 2014, including a rise in the incidence of clade B infections. The lack of association between AFM cases and EV-D68 in the US in 2015 and 2016 [11] does not detract from our analysis, as AFM is a clinical syndrome with multiple possible aetiologies. More recent AFM cases could be due to a different aetiology, as other EVs continue to cause AFM, or could reflect the difficulty in isolating the virus from tissue and CSF. The Bradford Hill criteria are a tested and systematic method for evaluating causality and could be applied to other EVs.

Conclusion

Go to section...

In summary, the application of the Bradford Hill criteria suggests that EV-D68 causes AFM. AFM has not previously been associated with EV-D68, and a mouse model shows that the original Fermon strain does not cause AFM, whereas the 2014 outbreak strain does [47]. It appears that the incidence of this infection and the clade-specific epidemiology have changed. Phylogeographic epidemiology will further our understanding of the temporal and spatial spread of increasingly neurovirulent clades and improve risk analysis. Further investigation into this relationship is important because of the severity of AFM, ongoing outbreaks of AFM and because there is currently no treatment for AFM related to EV-D68, and no vaccine to prevent infection [24,65].

Acknowledgements

Go to section...

Funding statement: The work was funded by NHMRC Centre for Research Excellence, Integrated Systems for Epidemic Response (ISER) (grant #1107393).

Data availability statement: All data in this review are publicly available.

Conflict of interest None declared.

Authors' contributions AD: Contributed to the initial literature review and drafting of manuscript; SSB, DCA: Contributed to subsequent literature review, drafting and revision of the manuscript; AAC: Contributed to revision of the manuscript; CRM: Conception and design of the study, drafting and revision of the manuscript.

References

References

Go to section...

1. The Lancet Infectious Diseases. Enterovirus D68: the unexpected guest. Lancet Infect Dis. 2014;14(11):1023. https://doi.org/10.1016/S1473-3099(14)70968-5 PMID: 25444395
2. Centers for Disease Control and Prevention (CDC). Clusters of acute respiratory illness associated with human enterovirus 68 - Asia, Europe, and United States, 2008-2010. MMWR Morb Mortal Wkly Rep. 2011;60(38):1301-4.

3. Holm-Hansen CC, Midgley SE, Fischer TK. Global emergence of enterovirus D68: a systematic review. Lancet Infect Dis. 2016;16(5):e64-75. https://doi.org/10.1016/S1473-3099(15)00543-5 PMID: 26929196
4. Tang J, Tao Z, Ding Z, Zhang Y, Zhang J, Tian B, et al. Complete genome characterization of a novel enterovirus type EV-B106 isolated in China, 2012. Sci Rep. 2014;4(1):4255. https://doi.org/10.1038/srep04255 PMID: 24584702
5. Huang HI, Shih SR. Neurotropic enterovirus infections in the central nervous system. Viruses. 2015;7(11):6051-66. https://doi.org/10.3390/v7112920 PMID: 26610549
6. International Committee on Taxonomy of Viruses, King AMQ. Virus taxonomy: classification and nomenclature of viruses: ninth report of the International Committee on Taxonomy of Viruses. London: Elsevier; 2012.
7. Australian Paediatric Surveillance Unit. Study protocol: acute flaccid paralysis. Canberra: Commonwealth Department of Health; 2014. Available from: http://www.apsu.org.au/assets/current-studies/AFP-Study-Protocol-June-2014.pdf
8. World Health Organisation (WHO). Poliomyelitis. Geneva: WHO; 2017. Available from: http://www.who.int/mediacentre/factsheets/fs114/en/
9. World Health Organisation (WHO). Global polio eradication initiative. Geneva: WHO. [Accessed: 5 Jan 2018]. Available from: http://polioeradication.org/
10. Huang CC, Liu CC, Chang YC, Chen CY, Wang ST, Yeh TF. Neurologic complications in children with enterovirus 71 infection. N Engl J Med. 1999;341(13):936-42. https://doi.org/10.1056/NEJM199909233411302 PMID: 10498488
11. Centers for Disease Control and Prevention (CDC). AFM in the United States. Atlanta: CDC; 2017. Available from: http://www.cdc.gov/acute-flaccid-myelitis/afm-surveillance.html
12. Greninger AL, Naccache SN, Messacar K, Clayton A, Yu G, Somasekar S, et al. A novel outbreak enterovirus D68 strain associated with acute flaccid myelitis cases in the USA (2012-14): a retrospective cohort study. Lancet Infect Dis. 2015;15(6):671-82. https://doi.org/10.1016/S1473-3099(15)70093-9 PMID: 25837569
13. Centers for Disease Control and Prevention (CDC). Enterovirus D68. Atlanta: CDC; 2017. Available from: http://www.cdc.gov/non-polio-enterovirus/about/ev-d68.html
14. Khetsuriani N, Lamonte-Fowlkes A, Oberst S, Pallansch MA. Centers for Disease Control and Prevention. Enterovirus surveillance - United States, 1970-2005. MMWR Surveill Summ. 2006;55(8):1-20.
15. Tokarz R, Firth C, Madhi SA, Howie SRC, Wu W, Sall AA, et al. Worldwide emergence of multiple clades of enterovirus 68. J Gen Virol. 2012;93(Pt 9):1952-8. https://doi.org/10.1099/vir.0.043935-0 PMID: 22694903
16. Holm-Hansen CC, Midgley SE, Fischer TK. Global emergence of enterovirus D68: a systematic review. Lancet Infect Dis. 2016;16(5):e64-75. https://doi.org/10.1016/S1473-3099(15)00543-5 PMID: 26929196
17. Knoester M, Schölvinck EH, Poelman R, Smit S, Vermont CL, Niesters HG, et al. Upsurge of Enterovirus D68, the Netherlands, 2016. Emerg Infect Dis. 2017;23(1):140-3. https://doi.org/10.3201/eid2301.161313 PMID: 27660916

18. Böttcher S, Prifert C, Weißbrich B, Adams O, Aldabbagh S, Eis-Hübinger AM, et al. Detection of enterovirus D68 in patients hospitalised in three tertiary university hospitals in Germany, 2013 to 2014. Euro Surveill. 2016;21(19):30227. https://doi.org/10.2807/1560-7917.ES.2016.21.19.30227 PMID: 27195917
19. Lam HY, Wong AT, Tsao YC, Tang BS. Prevalence and phylogenetic characterization of human enterovirus D68 among children with respiratory infection in Hong Kong. Diagn Microbiol Infect Dis. 2016;85(2):174-6. https://doi.org/10.1016/j.diagmicrobio.2016.02.024 PMID: 27036976
20. Schuffenecker I, Mirand A, Josset L, Henquell C, Hecquet D, Pilorgé L, et al. Epidemiological and clinical characteristics of patients infected with enterovirus D68, France, July to December 2014. Euro Surveill. 2016;21(19):30226. https://doi.org/10.2807/1560-7917.ES.2016.21.19.30226 PMID: 27195770
21. Zhang T, Li A, Chen M, Wu J, Huang F. Respiratory infections associated with enterovirus D68 from 2011 to 2015 in Beijing, China. J Med Virol. 2016;88(9):1529-34. https://doi.org/10.1002/jmv.24505 PMID: 26896830
22. Disease debrief: EV-D68. Winnipeg: National Collaborating Centre for Infectious Diseases; 2015. Available from: https://nccid.ca/debrief/ev-d68/
23. Peci A, Winter AL, Warshawsky B, Booth TF, Eshaghi A, Li A, et al. Epidemiology of enterovirus D68 in Ontario. PLoS One. 2015;10(11):e0142841. https://doi.org/10.1371/journal.pone.0142841 PMID: 26599365
24. Tyler KL. Rationale for the evaluation of fluoxetine in the treatment of enterovirus D68-associated acute flaccid myelitis. JAMA Neurol. 2015;72(5):493-4. https://doi.org/10.1001/jamaneurol.2014.4625 PMID: 25775436
25. Sejvar JJ, Lopez AS, Cortese MM, Leshem E, Pastula DM, Miller L, et al. Acute flaccid myelitis in the United States, August-December 2014: results of nationwide surveillance. Clin Infect Dis. 2016;63(6):737-45. https://doi.org/10.1093/cid/ciw372
26. Bragstad K, Jakobsen K, Rojahn AE, Skram MK, Vainio K, Holberg-Petersen M, et al. High frequency of enterovirus D68 in children hospitalised with respiratory illness in Norway, autumn 2014. Influenza Other Respi Viruses. 2015;9(2):59-63. https://doi.org/10.1111/irv.12300 PMID: 25534826
27. Bragstad K, Jakobsen K, Rojahn AE, Skram MK, Vainio K, Holberg-Petersen M, et al. High frequency of enterovirus D68 in children hospitalised with respiratory illness in Norway, autumn 2014. Influenza Other Respi Viruses. 2015;9(2):59-63. https://doi.org/10.1111/irv.12300 PMID: 25534826
28. Pfeiffer HCBK, Bragstad K, Skram MK, Dahl H, Knudsen PK, Chawla MS, et al. Two cases of acute severe flaccid myelitis associated with enterovirus D68 infection in children, Norway, autumn 2014. Euro Surveill. 2015;20(10):21062. https://doi.org/10.2807/1560-7917.ES2015.20.10.21062 PMID: 25788251
29. Lang M, Mirand A, Savy N, Henquell C, Maridet S, Perignon R, et al. Acute flaccid paralysis following enterovirus D68 associated pneumonia, France, 2014. Euro

Surveill. 2014;19(44):20952. https://doi.org/10.2807/1560-7917.ES2014.19.44.20952 PMID: 25394254

30. European Centre for Disease Control and Prevention (ECDC). Rapid risk assessment. Enterovirus D68 detected in the USA, Canada and Europe. Second update. Stockholm: ECDC; 25 November 2014. Available from: https://ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/Enterovirus-68-detected-in-the-USA-Canada-Europe-second-update-25-November-2014.pdf
31. Du J, Zheng B, Zheng W, Li P, Kang J, Hou J, et al. Analysis of enterovirus 68 strains from the 2014 North American outbreak reveals a new clade, indicating viral evolution. PLoS One. 2015;10(12):e0144208. https://doi.org/10.1371/journal.pone.0144208 PMID: 26630383
32. Gong YN, Yang SL, Shih SR, Huang YC, Chang PY, Huang CG, et al. Molecular evolution and the global reemergence of enterovirus D68 by genome-wide analysis. Medicine (Baltimore). 2016;95(31):e4416. https://doi.org/10.1097/MD.0000000000004416 PMID: 27495059
33. Hill AB. The environment and disease: association or causation? Proc R Soc Med. 1965;58:295-300. PMID: 14283879
34. Aliabadi N, Messacar K, Pastula DM, Robinson CC, Leshem E, Sejvar JJ, et al. Enterovirus D68 infection in children with acute flaccid myelitis, Colorado, USA, 2014. Emerg Infect Dis. 2016;22(8):1387-94. https://doi.org/10.3201/eid2208.151949 PMID: 27434186
35. Willyard C. An unknown enemy: drugs sought against EV-68 as paralysis link is explored. Nat Med. 2015;21(5):419-21. PMID: 25822590
36. Pastula DM, Aliabadi N, Haynes AK, Messacar K, Schreiner T, Maloney J, et al. Acute neurologic illness of unknown etiology in children - Colorado, August-September 2014. MMWR Morb Mortal Wkly Rep. 2014;63(40):901-2. PMID: 25299607
37. Messacar K, Schreiner TL, Maloney JA, Wallace A, Ludke J, Oberste MS, et al. A cluster of acute flaccid paralysis and cranial nerve dysfunction temporally associated with an outbreak of enterovirus D68 in children in Colorado, USA. Lancet. 2015;385(9978):1662-71. https://doi.org/10.1016/S0140-6736(14)62457-0 PMID: 25638662
38. Van Haren K, Ayscue P, Waubant E, Clayton A, Sheriff H, Yagi S, et al. Acute flaccid myelitis of unknown etiology in California, 2012-2015. JAMA. 2015;314(24):2663-71. https://doi.org/10.1001/jama.2015.17275 PMID: 26720027
39. Ayscue P, Van Haren K, Sheriff H, Waubant E, Waldron P, Yagi S, et al. . Acute flaccid paralysis with anterior myelitis - California, June 2012-June 2014. MMWR Morb Mortal Wkly Rep. 2014;63(40):903-6. PMID: 25299608
40. Levy A, Roberts J, Lang J, Tempone S, Kesson A, Dofai A, et al. Enterovirus D68 disease and molecular epidemiology in Australia. J Clin Virol. 2015;69:117-21. https://doi.org/10.1016/j.jcv.2015.06.079 PMID: 26209392

41. Crone M, Tellier R, Wei XC, Kuhn S, Vanderkooi OG, Kim J, et al. Polio-like illness associated with outbreak of upper respiratory tract infection in children. J Child Neurol. 2016;31(4):409-14. https://doi.org/10.1177/0883073815596613 PMID: 26215391
42. Schieble JH, Fox VL, Lennette EH. A probable new human picornavirus associated with respiratory diseases. Am J Epidemiol. 1967;85(2):297-310. https://doi.org/10.1093/oxfordjournals.aje.a120693 PMID: 4960233
43. Hashimoto I, Hagiwara A. Pathogenicity of a poliomyelitis-like disease in monkeys infected orally with enterovirus 71: a model for human infection. Neuropathol Appl Neurobiol. 1982;8(2):149-56. https://doi.org/10.1111/j.1365-2990.1982.tb00269.x PMID: 7048123
44. Victorio CB, Xu Y, Ng Q, Chua BH, Alonso S, Chow VT, et al. A clinically authentic mouse model of enterovirus 71 (EV-A71)-induced neurogenic pulmonary oedema. Sci Rep. 2016;6(1):28876. https://doi.org/10.1038/srep28876 PMID: 27357918
45. Wang YF, Yu CK. Animal models of enterovirus 71 infection: applications and limitations. J Biomed Sci. 2014;21(1):31. https://doi.org/10.1186/1423-0127-21-31 PMID: 24742252
46. Vollbach S, Müller A, Drexler JF, Simon A, Drosten C, Eis-Hübinger AM, et al. Prevalence, type and concentration of human enterovirus and parechovirus in cerebrospinal fluid samples of pediatric patients over a 10-year period: a retrospective study. Virol J. 2015;12(1):199. https://doi.org/10.1186/s12985-015-0427-9 PMID: 26607060
47. Volle R, Bailly JL, Mirand A, Pereira B, Marque-Juillet S, Chambon M, et al. Variations in cerebrospinal fluid viral loads among enterovirus genotypes in patients hospitalized with laboratory-confirmed meningitis due to enterovirus. J Infect Dis. 2014;210(4):576-84. https://doi.org/10.1093/infdis/jiu178 PMID: 24652491
48. Hixon AM, Yu G, Leser JS, Yagi S, Clarke P, Chiu CY, et al. A mouse model of paralytic myelitis caused by enterovirus D68. PLoS Pathog. 2017;13(2):e1006199. https://doi.org/10.1371/journal.ppat.1006199 PMID: 28231269
49. McMinn PC. Recent advances in the molecular epidemiology and control of human enterovirus 71 infection. Curr Opin Virol. 2012;2(2):199-205. https://doi.org/10.1016/j.coviro.2012.02.009 PMID: 22482716
50. Yamayoshi S, Yamashita Y, Li J, Hanagata N, Minowa T, Takemura T, et al. Scavenger receptor B2 is a cellular receptor for enterovirus 71. Nat Med. 2009;15(7):798-801. https://doi.org/10.1038/nm.1992 PMID: 19543282
51. Bek EJ, McMinn PC. The pathogenesis and prevention of encephalitis due to human enterovirus 71. Curr Infect Dis Rep. 2012;14(4):397-407. https://doi.org/10.1007/s11908-012-0267-3 PMID: 22639066
52. Fujii K, Nagata N, Sato Y, Ong KC, Wong KT, Yamayoshi S, et al. Transgenic mouse model for the study of enterovirus 71 neuropathogenesis. Proc Natl Acad Sci USA. 2013;110(36):14753-8. https://doi.org/10.1073/pnas.1217563110 PMID: 23959904

53. Liu Y, Sheng J, Baggen J, Meng G, Xiao C, Thibaut HJ, et al. Sialic acid-dependent cell entry of human enterovirus D68. Nat Commun. 2015;6:8865. https://doi.org/10.1038/ncomms9865 PMID: 26563423
54. Xiang Z, Li L, Lei X, Zhou H, Zhou Z, He B, et al. Enterovirus 68 3C protease cleaves TRIF to attenuate antiviral responses mediated by Toll-like receptor 3. J Virol. 2014;88(12):6650-9. https://doi.org/10.1128/JVI.03138-13 PMID: 24672048
55. Kreuter JD, Barnes A, McCarthy JE, Schwartzman JD, Oberste MS, Rhodes CH, et al. A fatal central nervous system enterovirus 68 infection. Arch Pathol Lab Med. 2011;135(6):793-6. PMID: 21631275
56. Pérez-Vélez CM, Anderson MS, Robinson CC, McFarland EJ, Nix WA, Pallansch MA, et al. Outbreak of neurologic enterovirus type 71 disease: a diagnostic challenge. Clin Infect Dis. 2007;45(8):950-7. https://doi.org/10.1086/521895 PMID: 17879907
57. Modlin JF. Enterovirus déjà vu. N Engl J Med. 2007;356(12):1204-5. https://doi.org/10.1056/NEJMp078016 PMID: 17377157
58. Komatsu H, Shimizu Y, Takeuchi Y, Ishiko H, Takada H. Outbreak of severe neurologic involvement associated with Enterovirus 71 infection. Pediatr Neurol. 1999;20(1):17-23. https://doi.org/10.1016/S0887-8994(98)00087-3 PMID: 10029254
59. Wang SM, Liu CC, Tseng HW, Wang JR, Huang CC, Chen YJ, et al. Clinical spectrum of enterovirus 71 infection in children in southern Taiwan, with an emphasis on neurological complications. Clin Infect Dis. 1999;29(1):184-90. https://doi.org/10.1086/520149 PMID: 10433583
60. Xiang Z, Xie Z, Liu L, Ren L, Xiao Y, Paranhos-Baccalà G, et al. Genetic divergence of enterovirus D68 in China and the United States. Sci Rep. 2016;6(1):27800. https://doi.org/10.1038/srep27800 PMID: 27278628
61. Ayukekbong JA, Bergström T. Polio will go, acute flaccid paralysis will stay. Lancet. 2014;383(9936):2209-10. https://doi.org/10.1016/S0140-6736(14)61080-1 PMID: 24976324
62. da Silva EE, Winkler MT, Pallansch MA. Role of enterovirus 71 in acute flaccid paralysis after the eradication of poliovirus in Brazil. Emerg Infect Dis. 1996;2(3):231-3. https://doi.org/10.3201/eid0203.960312 PMID: 8903236
63. Brasil P, Pereira JP Jr, Moreira ME, Ribeiro Nogueira RM, Damasceno L, Wakimoto M, et al. Zika virus infection in pregnant women in Rio de Janeiro. N Engl J Med. 2016;375(24):2321-34. https://doi.org/10.1056/NEJMoa1602412 PMID: 26943629
64. Kleber de Oliveira W, Cortez-Escalante J, De Oliveira WT, do Carmo GM, Henriques CM, Coelho GE, et al. Increase in reported prevalence of microcephaly in infants born to women living in areas with confirmed Zika virus transmission during the first trimester of pregnancy - Brazil, 2015. MMWR Morb Mortal Wkly Rep. 2016;65(9):242-7. https://doi.org/10.15585/mmwr.mm6509e2 PMID: 26963593
65. Cauchemez S, Besnard M, Bompard P, Dub T, Guillemette-Artur P, Eyrolle-Guignot D, et al. Association between Zika virus and microcephaly in French Polynesia, 2013-2015: a retrospective study. Lancet. 2016;387(10033):2125-32. https://doi.org/10.1016/S0140-6736(16)00651-6
66. Liu Y, Sheng J, Fokine A, Meng G, Shin WH, Long F, et al. Structure and inhibition of EV-D68, a virus that causes respiratory illness in children. Science.

2015;347(6217):71-4. https://doi.org/10.1126/science.1261962 PMID: 25554786

EXHIBIT NO.

20

Frontiers

In Microbiology

Front Microbol 2018:9:2607 PMCID: PMC 6243117

Published on line 2018 Nov. 13 doi. 10:3389/ FMICH.2018.02677

PMID: 30483226

Enterovirus D68 – The New Polio?

Hayley Cassidy, Randy Poelman, Marjolein Knoester, Coretta C. Van Leer-Buter, and Hubert G. M. Niesters*

Author information Article notes Copyright and License information Disclaimer

Abstract

Go to:

Introduction

Enterovirus D68: The Virus

Enterovirus D68 is a single-stranded positive-sense RNA virus of the *Picornaviridae* family, belonging to the species enterovirus D. EV-D68 was first isolated from respiratory samples in 1962 in California, United States from four pediatric patients presenting with acute respiratory symptoms. The four isolates obtained from the patients were referred to as the Fermon, Franklin, Robinson,and Rhyne strains, each presenting with similar antigenic properties. As a representative strain of the new serotype, the Fermon strain was selected (Imamura and Oshitani, 2015). Since its first description, EV-D68 has been classified into three genetic clades, A, B and C. Subclades A1, A2, B1, and B2 have evolved and can be further identified depending on enterovirus typing, targeting either VP1 or VP4-2 capsid protein (Nix et al., 2006; Esposito et al., 2015). EV-D68 was also previously known as rhinovirus 87 until it was re-classified in 2002 (Blomqvist et al., 2002). EV-D68 is unusual in that it has shared characteristics from two key members of the *Picornaviridae* family; enterovirus and rhinovirus. Firstly, it has a lower optimal growth temperature of 33°C (the temperature of the nose), allowing better replication in the nasal cavity than other EV, and secondly it is acid sensitive, meaning it is unable to adequately survive

during passage in the stomach (Foster et al., 2015). Phylogeny however, shows that EV-D68 is genetically more closely related to EV than to rhinoviruses. Studies into the cellular receptors of EV-D68 have shown that it targets the α2-6-linked sialic acid, which is present on cells in the upper respiratory tract, indicating tropism toward this area (Imamura et al., 2014). This critical difference in tropism is significant when comparing EV-D68 to poliovirus, which predominantly reproduces in the gastrointestinal tract. It is likely that most EV-D68 infections are asymptomatic or present with a mild respiratory illness however, it is difficult to know it's true circulation and burden on the community. Nevertheless, studies have indicated that circulation of EV-D68 increases over the summer-autumn season like with other EV (Tokarz et al., 2012).

The Beginning of Enterovirus Awareness – The Polio Era

Enteroviruses are thought to have existed and coevolved with humanity for thousands of years. One of the oldest records of enterovirus is an Egyptian carving thought to illustrate a priest with a small, weakened limb, which is considered a typical feature of a past polio infection. The causative agent of poliomyelitis (poliovirus), was not discovered until 1908 (Paul, 1971). It was not only the first enterovirus to be discovered, but also caused the most devastating and widespread morbidity and mortality of all the enterovirus genotypes. Poliovirus infection can result in a variety of symptoms, of which AFP that can cause lifelong disability and may result in death, is the most typical clinical entity. Awareness of polio increased during the 20th century. This is largely attributed to President Franklin Roosevelt, himself paralyzed from polio, who was instrumental in founding the National Foundation for Infantile Paralysis which started mass worldwide vaccination campaigns (Noor and Krilov, 2016). The first poliovirus vaccine was an inactivated injectable vaccine, developed by Jonas Salk in 1955. The second vaccine administered orally, was a vaccine developed by Albert Sabin in 1961 (Noor and Krilov, 2016). These primary awareness programs, together with the vaccination campaign, initiated in the fifties, paved the way for the GPEI created in 1988, which aimed to eradicate the virus. Over the subsequent years, following the initial discovery of poliovirus, over 100 enterovirus serotypes have now been discovered with nearly 70 species infecting humans (Craighead, 2000). Non-polio EV can cause a variety of clinical syndromes, ranging from hand-foot and mouth disease to aseptic meningitis.

sThe introduction of the poliovirus vaccine dramatically reduced the incidence of infections globally, with only small clusters sporadically occurring. According to the GPEI, only two countries, Afghanistan and Pakistan still report endemic wild-type poliovirus in circulation in 2018 (The Global Poliovirus Eradication Initiative

Vaccine ended 1/2 lo

[GPEI], 2018). Recently, the GPEI also reported a few new cases of vaccine-derived poliovirus in the Democratic Republic of the Congo, Nigeria, Somalia and Papua New Guinea (The Global Poliovirus Eradication Initiative [GPEI], 2018). Therefore, cases of AFP outside these countries have decreased to very low numbers. Remaining cases of AFP are also linked to GBS and neurological infections caused by other viruses such as WNV and more recently, non-polio EV.

The Rise in Awareness of EV-D68

In the majority of patients, EV-D68 only causes mild respiratory illness. However, the co-occurrence of EV-D68 and a predominantly severe respiratory disease on one side and neurological complications of "polio-like" paralysis on the other side has established EV-D68 as an emerging pathogen (Holm-Hansen et al., 2016). While EV-D68 has been known as a respiratory pathogen since its first description in 1962, the apparent change in pathogenicity into a virus capable of causing AFP over a relatively short period of time, has led to increased interest and awareness of the virus in recent years. This is reflected in the number of published papers. Figure Figure1 1 reveals the result of a PubMed search for Enterovirus-D68. With the disappearance of poliovirus as a major threat to public health, enterovirus networks such as the ENPEN, have been set up along with established networks such as the ESCV to focus on non-polio EV which may become new challenges (Harvala et al., 2018). EV-D68 has become a compelling topic for research over the recent years, due to a mix of increased prevalence, pathology and awareness. This review will explore the EV-D68 story further.

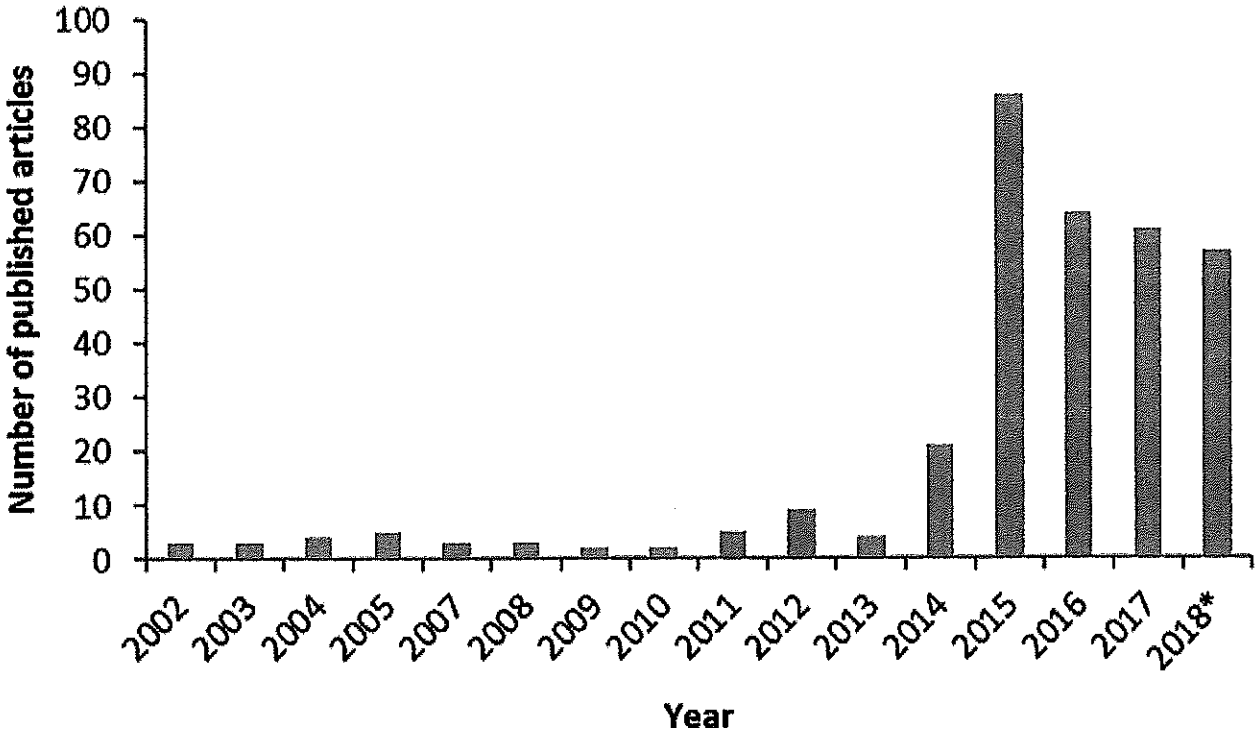


FIGURE 1

The number of published articles on PubMed describing Enterovirus-D68 from January 2002 to October2018. The number of published articles for each year is in accordance with EV-D68 interest from the previous year. *Until October.

Epidemiology: From Sporadic Respiratory Virus to Emerging Neuropathogenic Threat

Enterovirus D68 had only been reported sporadically worldwide since 2010. Indeed before 2014, only sporadic outbreaks were reported in the US, the Philippines, Japan and the Netherlands (Rahamat-Langendoen et al., 2011; Gong et al., 2016). Significantly, there were only 26 cases reported in the United States between 1970 and 2005 and only 699 cases described in Europe, Southeast Asia and Africa between 1970 and 2013 (Holm-Hansen et al., 2016; Wang et al., 2017). However, it must be noted that specific testing for EV-D68 or routine typing for EV was and is not standard practice in the majority of laboratories and assuredly not for testing respiratory samples. Therefore the true burden of disease is not known and trends have been likely to be missed. A study published in 2012 reports the analysis of trends in EV-D68 circulation across the United States and in Africa over two decades (Tokarz et al., 2012). This was a direct result of increasing sporadic reports of respiratory disease associated with EV-D68 across North America, Europe and Asia. The study found the genome had undergone a

rearrangement from the initial Fermon strain in the spacer region of the 5′UTR, which is known to affect the translational efficiency and thought to increase the virulence. Further rearrangement led to a separation into clades A, B and C, with additional deletions within each clade. Interestingly, another study (Kaida et al., 2017) reported clade B had specific substitutions in the BC-loop, which is found on the surface canyon of the VP1 protein, known to have a role in antigenicity. This evolution may have had significant implications in the run up to the 2014 outbreak, but remains unclear so far.

The 2014 Outbreak

In August 2014, the United States and Canada experienced the first few cases of what resulted in the largest known EV-D68 outbreak in history. An unprecedented number of cases, particularly in young children, of severe-respiratory illness was linked to EV-D68 infections. Unexpectedly, this outbreak of severe respiratory infections coincided with an upsurge in AFP. Many of the affected children were also shown to have concurrent EV-D68 infections. The Polio and Picornavirus Laboratory Branch of the CDC tested 56 respiratory samples and found EV-D68 to be the most commonly detected pathogen, with an overall rate of 20% (11/56) in samples tested. Furthermore the detection increased to 47% (8/17) in samples collected near onset of respiratory illness (≤7 days) (Sejvar et al., 2016). Fifty-five CSF specimens were also tested and found to have one positive EV-D68 sample (also positive for Epstein-Barr virus) (Sejvar et al., 2016). The paralysis seen in patients infected with EV-D68 was clinically defined as AFM which was essentially, an acute onset of AFP with MRI scans showing motor neuron damage in the myelum. This will be discussed more in detail later in see section "EV-D68 Case Definition." In 2014, in the United States alone, 120 children were reported with AFM which met the case definition (Messacar et al., 2016). Furthermore, over a 1000 hospital admissions and 12 deaths were associated with an EV-D68 respiratory infection (Levy et al., 2015). A subsequent study found that most EV-D68 positive samples associated with AFM, clustered into the B1 subclade. Interestingly, the study found that five out of six coding polymorphisms present in the subclade B1 strains were associated with neuropathogenic poliovirus (Greninger et al., 2015). These results suggested that the virus had changed in pathogenicity since the originally isolated Fermon strain, a hypothesis which was also supported by results of studies in mice models (Hixon et al., 2017b), which will be discussed later.

At that time, severe respiratory outbreaks of EV-68 were reported from several countries across Europe in 2014. During these outbreaks, cases of AFM were identified, including one in France and in the United Kingdom and two in Norway

(Lang et al., 2014; Pfeiffer et al., 2015; Varghese et al., 2015; Holm-Hansen et al., 2016). It is still unclear how frequently EV-D68 causes AFM compared to the number of respiratory infections caused by this virus. Estimating this frequency is currently impossible, firstly as the background circulation of this relatively emerging virus is unknown and secondly, only children with severe respiratory illness were tested in the 2014 outbreak in the United States and in Canada, therefore there is no information on the frequency at which EV-D68 causes mild symptoms. One study investigated EV-D68 detection by country, mostly in Northern and Western Europe, during the United States outbreak (Poelman et al., 2015b). Out of 17,248 respiratory (majority) specimens tested, 4273 had confirmed picornavirus detection with 389 samples positive for EV-D68. In Southern and Eastern Europe, too few samples were tested to draw any conclusions. Hence, crucial information about how large the threat of EV-D68 could be, is still missing.

Regarding poliomyelitis, studies have shown that 1 in 200 poliovirus infections led to irreversible paralysis, with 5–10% of paralysis cases resulting in death due to breathing difficulties (World Health Organization [WHO], 2018). As AFP is not a reportable disease in many countries, as long as it is not caused by poliovirus, it is impossible to say how many potential cases of EV-D68 associated AFM have occurred during upsurges of EV-D68 in the past few years. As the link between EV-D68 and AFM had not been established at that time, many children who presented with sudden paralysis were not adequately sampled to detect EV-D68. Subsequent data from the EV-D68 outbreak in the United States and Canada in 2014 indicates that neurological complications could occur in 1 out of 100 symptomatic cases with a total of 1153 confirmed EV-D68 respiratory infections, and 12 EV-D68 positive AFM cases during this period (Sejvar et al., 2016). Similarly, in Europe, out of 389 confirmed positive samples in 2014, four AFM cases and one death were associated with EV-D68 (Poelman et al., 2015b; Varghese et al., 2015). Limited EV-D68 detection was seen both in the United States and in Europe in 2015 (Wang et al., 2017).

The 2016 Outbreak

In 2016, a new upsurge in the number of EV-D68 cases was first reported from the Netherlands. The majority of patients presented with severe respiratory illness, but one case of AFM was seen in a 4-year-old boy (Knoester et al., 2017). Phylogenetic analysis of the samples revealed a different clustering to the 2014 outbreak strains. Simultaneously, similar upsurges were described by groups in Norway, Denmark, Germany, France, Spain, Portugal, Sweden, Wales (United Kingdom), Scotland (United Kingdom) and in the United States (Dyrdak et al., 2016; ECDC, 2016; Wang et al., 2017). In addition, cases of EV-D68 were also

linked with AFM in Wales (2 cases), Scotland (5 cases), England (1 case), Sweden (3 cases), Italy (2 cases), Spain (3 cases), France (at least 1) and Argentina (15 cases) with strains clustering with a divergent B3 lineage (Antona et al., 2016; Dyrdak et al., 2016, ECDC, 2016; PAHO/WHO, 2016; Williams et al., 2016; Cabrerizo et al., 2017; Esposito et al., 2017; Giombini et al., 2017; Kirolos et al., 2017; Stacpoole et al., 2017). A subsequent surveillance study (Knoester et al., 2018) presented the results of a survey which found a total of 29 cases reported of EV-D68 infections associated with AFM in Europe in 2016. Interestingly, a higher number of EV-D68-associated AFM cases were reported in Europe in 2016 compared to 2014; 29 versus four cases in 2014. This could suggest the "new" circulating B3 subclade is more neuropathogenic or perhaps more transmissible than the B1 clade, which was most frequently reported in 2014. However, this most likely is due to increased surveillance and awareness established in 2014. In the United States, 149 AFM cases were reported in 2016, yet the exact number of AFM cases associated with EV-D68 is not known. Although there were fewer reported EV-D68 infections in 2016 in the United States overall, some AFM cases associated with EV-D68 were reported by Wang et al., 2017.

Diagnosing an Enterovirus D68 Infection

Sample Collection

Depending on the clinical picture, several diagnostic samples can and should be collected to detect EV, such as CSF, feces, respiratory material and serum/plasma (Harvala et al., 2018). As most EV are transmitted via the fecal-oral route and replicate in the intestine, high viral loads are usually present in feces, therefore an effective material for detection and genotyping. Genotyping or serotyping are necessary and mandatory in cases of AFP to exclude poliovirus, and stool samples are essential to achieve this. However, EV-D68 is not readily detected in fecal samples, in addition it has a rhinovirus-like replication cycle in the nasal cavity. Indeed, the recently published PAHO/WHO report now recommends including a respiratory sample if AFP is suspected (PAHO/WHO report, 2017). Animal models have shown that EV-D68 can also disseminate to the CNS by retrograde axonal transport (Morrey et al., 2018). Multiple material types should therefore be collected if a patient presents with CNS symptoms: stool, CSF, blood and respiratory samples (Harvala et al., 2018).

Molecular Testing for Pan-Enterovirus Detection

Molecular testing is recognized as the gold standard for diagnosing an enterovirus infection. RT-PCR targeting the 5′UTR region has been established as a routine

molecular test in many laboratories throughout Europe and other parts of the world (Harvala et al., 2018). It is used as a broad spectrum assay to detect the presence of EV, without regard to specific subtypes. The majority of assays are laboratory developed tests and can be used to screen a panel of suspected pathogens or to detect a specific target. Rapid and self-contained specimen-to-result tests such as the FilmArray system (BioFire/bioMerieux, Salt Lake City, United States) have also been used to screen and identify enterovirus /rhinovirus simultaneously by combining a nested multiplex PCR with melting curve analysis (Poritz et al., 2011). However, further tests would be needed as the Film-Array cannot distinguish enterovirus from rhinovirus. Additional FDA approved tests for enterovirus detection are the Cepheid GeneXpert and SmartCycler (Cepheid, Sunnyvale, California, United States). Finally, cell cultures can be used, however, they are not suitable for all enterovirus strains and further identification is required, using serotyping methods or real-time RT PCR.

A specific RT-PCR was developed to detect EV-D68 during a European surveillance project in 2014 (Poelman et al., 2015b). During an EV-D68 season (Summer-Autumn) or during an upsurge of the virus, a specific RT-PCR could be used for rapid diagnostics and patient management, and is particularly useful in the work-up of severe respiratory infections and AFM. During the 2014 outbreak, a study was carried out where the FilmArray system was used to detect positive enterovirus samples through its enterovirus/rhinovirus signals in a respiratory panel (Shibib et al., 2016). It was consequently found that a positive detection in the Rhinovirus 1 and 4 targets led to a high association (13.1× more likely) of EV-D68 found in subsequent samples sent to the CDC for confirmation. Further point of care tests were also evaluated during the outbreak. Similarly, the GenMark eSensor respiratory viral panel (Carlsbad, California, United States) was found to pick up low-positive rhinoviruses which were later found to be EV-D68 in 67% of samples, with a 94% sensitivity and 88% specificity rate (McAllister et al., 2015). This was subsequently noted due to cross-reactivity with rhinoviruses (McAllister et al., 2015; Diaz-Decaro et al., 2018).

Molecular Testing for Genotyping

Sanger sequencing of the VP1, and occasionally VP4-2 structural proteins, following detection of a positive EV sample is considered the gold standard for the determination of specific EV genotypes, according to World Health Organization (WHO) guidelines and Nix et al., 2006. Type specific RT-PCR and Sanger sequencing techniques have been used increasingly during outbreaks of EV-A71 in Asia and EV-D68 in the United States and Europe (Poelman et al., 2015a; Duong et al., 2016). Sequencing has transformed diagnostics, increasing the amount of

knowledge on pathogens, their circulation and phylogeny. Phylogenetic analysis can be used more efficiently to look for genetic relationships within the EV subtype. However, typing enterovirus remains difficult due to vast variations in the genome (Midgley et al., 2015).

The development of these molecular tests has also allowed for the rapid detection and reporting of results in real time. A steady flow of epidemiological data should be available from health agencies or regional centers and fed back to the performing labs, and vice versa. This is not always optimal, and improvements could be achieved through for example the Antimicrobial, Infection and Prevention and Diagnostic stewardship model (Dik et al., 2016).

Next Generation Sequencing

Next generation sequencing, in comparison to the Sanger targeted approach, allows for the sequencing of multiple reads at once by reading optical signals after each base addition (Escalona et al., 2016). Useful information about changes in tropism or pathogenicity has been obtained by NGS. A retrospective study into the diversity of EV-D68 during the United States outbreak in 2014 using NGS technologies (metagenomic shotgun sequencing) revealed specific polymorphisms C3277A and A4020G, which triggered functional mutations at cleavage sites $2A^{pro}$ and $3C^{pro}$ respectively (Huang et al., 2015). These amino acid substitutions are suspected to alter protease activity and increase replication and transmission rates. The group also found similar mutations in a 2013 strain (US/CO/13-60), thought to be an ancestor of the 2014 outbreak strains. Indeed these coding polymorphisms were found to be present in poliovirus, as mentioned previously (Huang et al., 2015).

Go to:

Ev-D68 Association With Acute Flaccid Myelitis (AFM)

The Strength of EV-D68 Association With AFM

The upsurge in infection and awareness has led to the expansion of current scientific knowledge, with several groups now investigating the depth of the association of EV-D68 with AFM. Many begin by describing the paralleling of numbers from the CDC for EV-D68 infections and the cases of AFM for 2014–2018 (Figure (Figure2;2; Dyda et al., 2018; Messacar et al., 2018). From looking at the literature, during and after the United States 2014 outbreak, similar upsurges of severe respiratory and neurological symptoms were found worldwide, most notably in Northern Europe. The association was further strengthened by a group

(Aliabadi et al., 2016) which found infection with EV-D68 resulted in a higher odds ratio than two control groups (10× and 4.5× respectfully) for AFM presentation. Furthermore, the respiratory prodromal phase prior to paralysis in 65% of patients (Martin et al., 2016), along with a high involvement of the cranial and spinal cord, appear to be more specifically associated with an EV-D68 infection (Messacar et al., 2018). Additionally, AFM mainly affects children, similarly to EV-D68, which indicates a specific target population (Dyda et al., 2018).

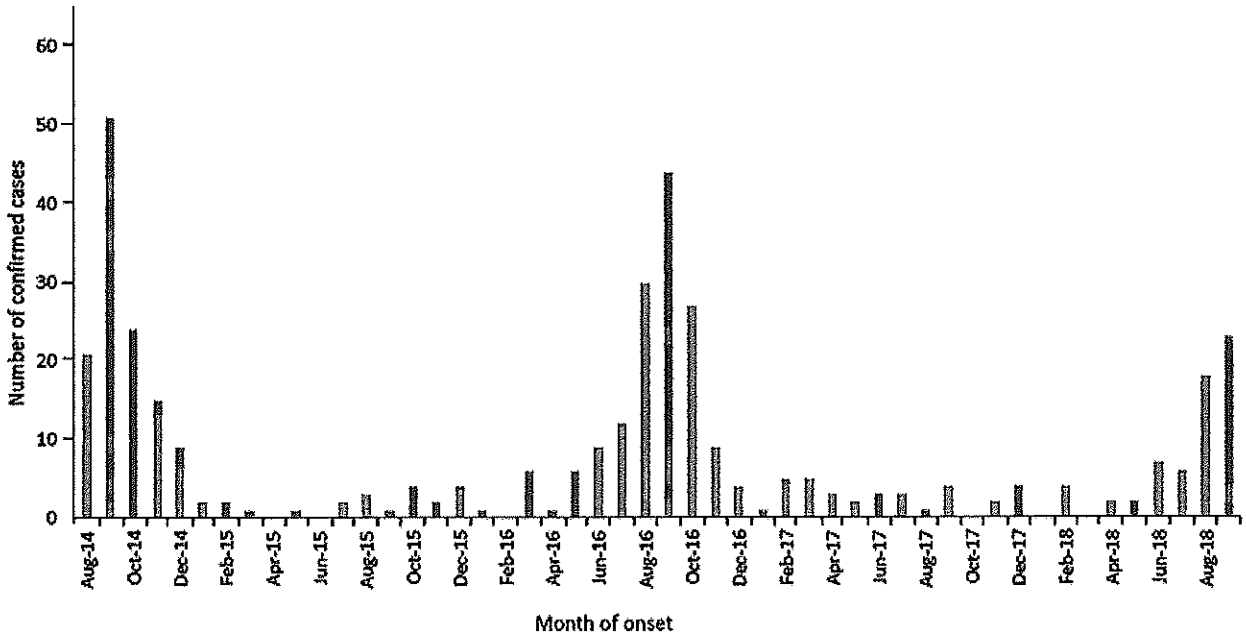


FIGURE 2

The number of confirmed United States AFM cases, published on the CDC by month of onset from August 2014-October 2018. The high number of confirmed AFM cases coincided with the 2014, 2016 and 2018 EV-D68 outbreaks. Figure taken from the NCIRD, AFM in the United States 2018. CDC and NCIRD (2018)retrieved from https://www.cdc.gov/acute-flaccid-myelitis/afm-surveillance.html.

One of the challenges of the association is that EV-D68 is not always detected in clinical samples from AFM patients. One group (Greninger et al., 2015) found EV-D68 in respiratory samples in approximately 48% of AFM patients (25 cases), where no other pathogen including EV-D68, could be detected in CSF samples. Indeed, as discussed previously, detection of EV-D68 in CSF is uncommon. This has only been reported in a handful of cases (Kreuter et al., 2011; Esposito et al., 2016; Giombini et al., 2017; Ruggieri et al., 2017). A recent paper (Morrey et al.,

2018) investigated this link through mouse models, deficient in interferon responses. Mice were challenged IP with EV-D68 of a known viral load. These researchers detected EV-D68 in the spinal cord just at early onset of paralysis, compared with the muscle injection site, which persisted for 6 weeks. The fact that the virus does not reside in the spinal cord for long could reflect the challenges in EV-D68 detection in CSF samples. Other viruses causing neurological disease including poliovirus, EV-A71, WNV and Rabies are similarly absent from CSF (Huang and Shih, 2015). Awareness of research in this area is particularly important for clinicians, as once an association is proven, they may be more inclined to take a respiratory sample and request for EV-D68 testing as well, during suspected AFM cases.

Experiments Revealing the Neurotropic Nature of EV-D68

The unprecedented upsurge in AFM associated with EV-D68 has led to many questions into why this emerging virus has become so pathogenic, leading to the polio-like paralysis observed. At present the exact mechanism which EV-D68 uses to instigate infection remains largely unknown. Studies have shown that poliovirus can gain access to the CNS through axonal transport and neuromuscular junctions (Ohka et al., 1998; Huang and Shih, 2015), and it could be possible that EV-D68 follows a similar mechanism. A recent study in an EV-D68 mouse model, links paralysis to infections with EV-D68 through intramuscular injection by using various strains of the virus, including the 2014 outbreak strains. These injections resulted in the loss of motor neurons in the anterior horn of the corresponding spinal cord segments, leading to paralyzed limbs. The study goes on to suggest that replication of the virus in the motor neurons causes the damage, paralleling the development of neurological symptoms (Hixon et al., 2017b). Significantly, the study fulfills Koch's postulates by activating paralysis in naïve mice from EV-D68 isolated from the spinal cord of a paralyzed mouse.

Much is still unknown about the exact mechanism EV-D68 uses to gain entry and replicate. Recently, a group investigating the life cycle of EV-D68 has started to answer some of the questions (Baggen et al., 2018). It was found that EV-D68 binds to synthetic glycoproteins through a terminally linked sialic acid. This binding induces a conformational change in the viral capsid, which commences the uncoating process, to inject the RNA and initiate the replication (Baggen et al., 2018). Sialic acid has been understood to be a binding site for other EV, such as Coxsackievirus A24, which was associated with acute haemorrhagic conjunctivitis (Baggen et al., 2018).

Wei et al. (2016) identified ICAM-5 as a possible entry receptor for EV-D68. EV-D68 was believed to bind to the ICAM-5-Fc receptor, where sialic acid was thought to induce a conformation change. Crucially, the telencephalon region of the brain was found to have enhanced ICAM-5 expression, this receptor could help explain the neurotropism of this virus (Wei et al., 2016; Messacar et al., 2018). Herpes Simplex Virus-1, another well-known neurotropic virus, also interacts with ICAM-5 to mediate cytokine secretion during infection (Tse et al., 2009). Although these studies shed a light on how EV-D68 may achieve its neuro-invasive capability, many more questions remain concerning the factors which determine the variability in disease severity which is seen in clinical cases. It is possible that changes in the genome, through mutational or selection pressure, led to an altered pathogenicity. Evidence in favor of this hypothesis has been shown by other authors, describing genogroup replacement between 2006 and 2014 (Xiang et al., 2016), and specific mutations in the puff region (key neutralization site) of VP2 in EV-D68 strains, which were isolated from patients with severe respiratory infections. Xiang et al. (2016) goes on to describe a mutational difference between the sequences obtained from the United States and China strains. In the United States strains there was a mutation in the pseudoknot structure in 3′-UTR, resulting in an altered phenotype comparatively to the Chinese strains. This could account for the differences in outbreaks between the United States and China in 2014.

EV-D68 Case Definition

Although EV-D68 has shown similar neurological presentation to poliovirus, as well as similar MRI features, it has its own specific case definition. In response to the increased number of severe respiratory infections and number of acute paralysis cases during the 2014 outbreak the CDC proposed the case definition; "*onset of acute limb weakness on or after August 1, 2014, and a magnetic resonance image (MRI) showing a spinal cord lesion largely restricted to gray matter in a patient age ≤ 21 years*" (Washington State Department of Health, 2016). AFP patients who presented with pleocytosis (white blood cells >5 mm^3) in their CSF who had a negative or no MRI result were recommended as a probable case. The terms AFM and AFP are used interchangeably in articles and reports from 2014. Adding to the confusion is the restriction of the AFM case definition to individuals younger than 21 years of age, as it is currently understood that AFM does occur in adults as well. Although mostly children with a chronic illness were affected, children and adults without any known underlying condition were also reported (Williams et al., 2016; Stacpoole et al., 2017). Additionally, it must be updated in accordance with increased data gained from reports.

The establishment of a case definition for AFM was particularly important as other diseases such as GBS, may also present with AFP. Although, paralysis tends to be more symmetrical in GBS (Jasti et al., 2016). Cases of EV-D68 associated AFM could be diagnosed as "atypical-GBS," unless an MRI scan is made or electromyography examinations are carried out. Typically, EV-D68 associated AFM develops following acute febrile respiratory syndrome, up to 2 weeks prior to onset of weakness (Tyler, 2015). Prodromal symptoms compatible with respiratory infections including shortness of breath (82%), cough (82%), and rhinorrhoea or nasal congestion (71%) (Martin et al., 2016) could be incorporated into the case definition.

Clinical Characteristics and Diagnosis of EV-D68 AFM

Patients presenting with a suspected EV-D68 associated AFM should undergo a series of examinations to confirm this diagnosis. One of the most valuable examinations, MRI, was used extensively during the 2014 outbreak to facilitate the establishment of a case definition. Patients can present with a variety of symptoms varying from cough, runny nose and diarrhea to muscles aches, fever and in some cases respiratory distress, particularly in children younger than five (National Center for Immunization and Respiratory Diseases [NCIRD], Division of Viral Diseases, 2017; Knoester et al., 2018). AFM symptoms are typically described as asymmetric motor weakness mostly affecting the upper limbs in the majority of current known cases. The weakness is flaccid, with deep-tendon reflexes reduced or absent (CDC and NCIRD, 2015). The cranial nerves are commonly affected with symptoms such as facial weakness, dysarthria and dysphagia being described (Tyler, 2015). Most patients did undergo a spinal tap on presentation, which showed cerebrospinal fluid pleocytosis in the majority of cases (Tyler, 2015). An MRI scan is essential for the diagnosis, as it shows lesions in the anterior horn of the gray matter along the spinal cord and sometimes in the brainstem (Tyler, 2015). As shown in the MRI images (Figures (Figures33,,4),4), these distinctive lesions point at the involvement of the spinal cord motor neurons. It must be noted that the lesions seen on the MRI scans during cases of EV-D68 associated AFM are identical to lesions in the spinal cord which are found in poliomyelitis. Diagnosing a case of AFM requires input from radiologists, clinical virologists, pediatricians and neurologists and highlights the need for communication between specialists to ensure a case is recognized, with the appropriate samples and tests requested, including PCR and MRI scans.

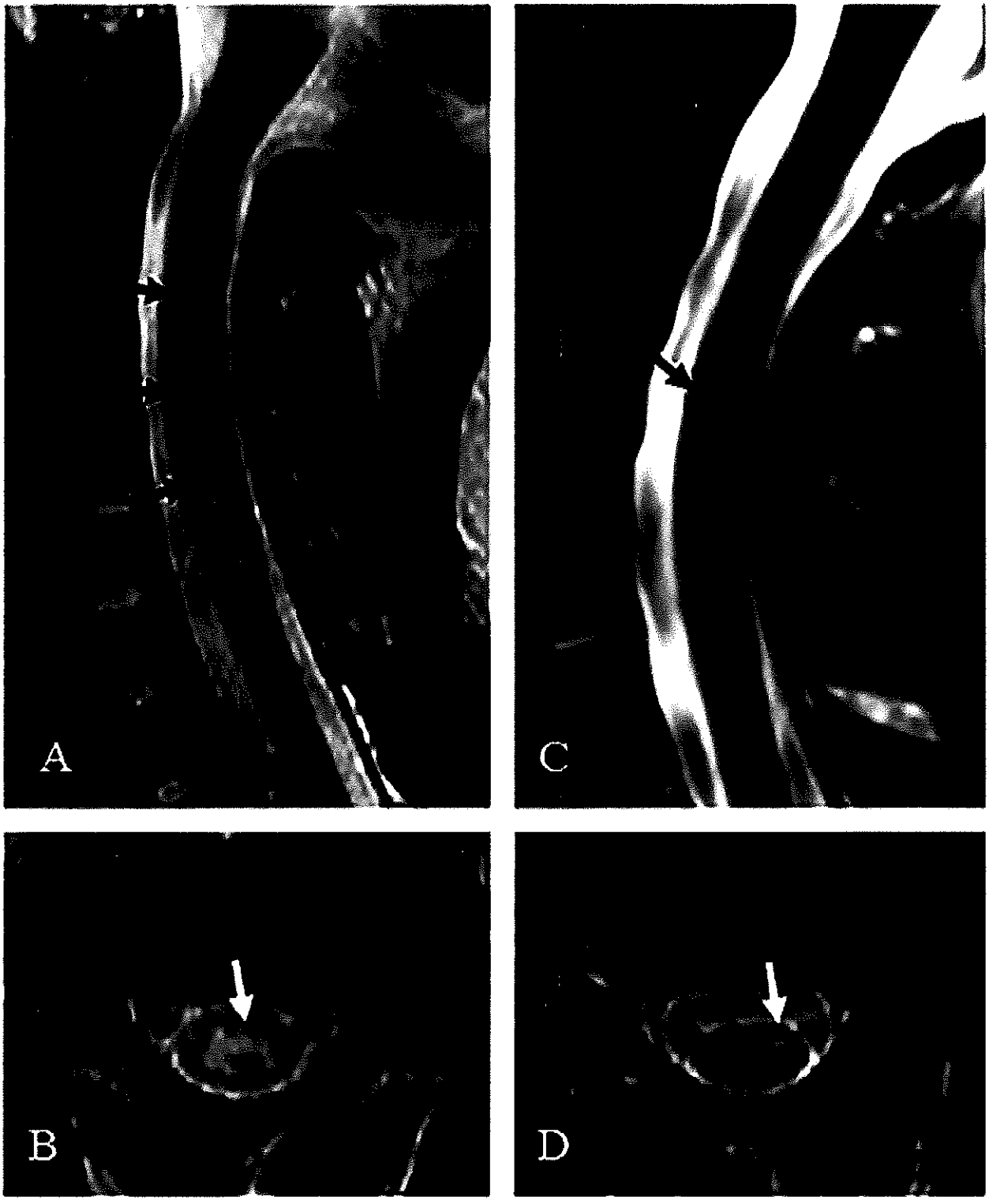
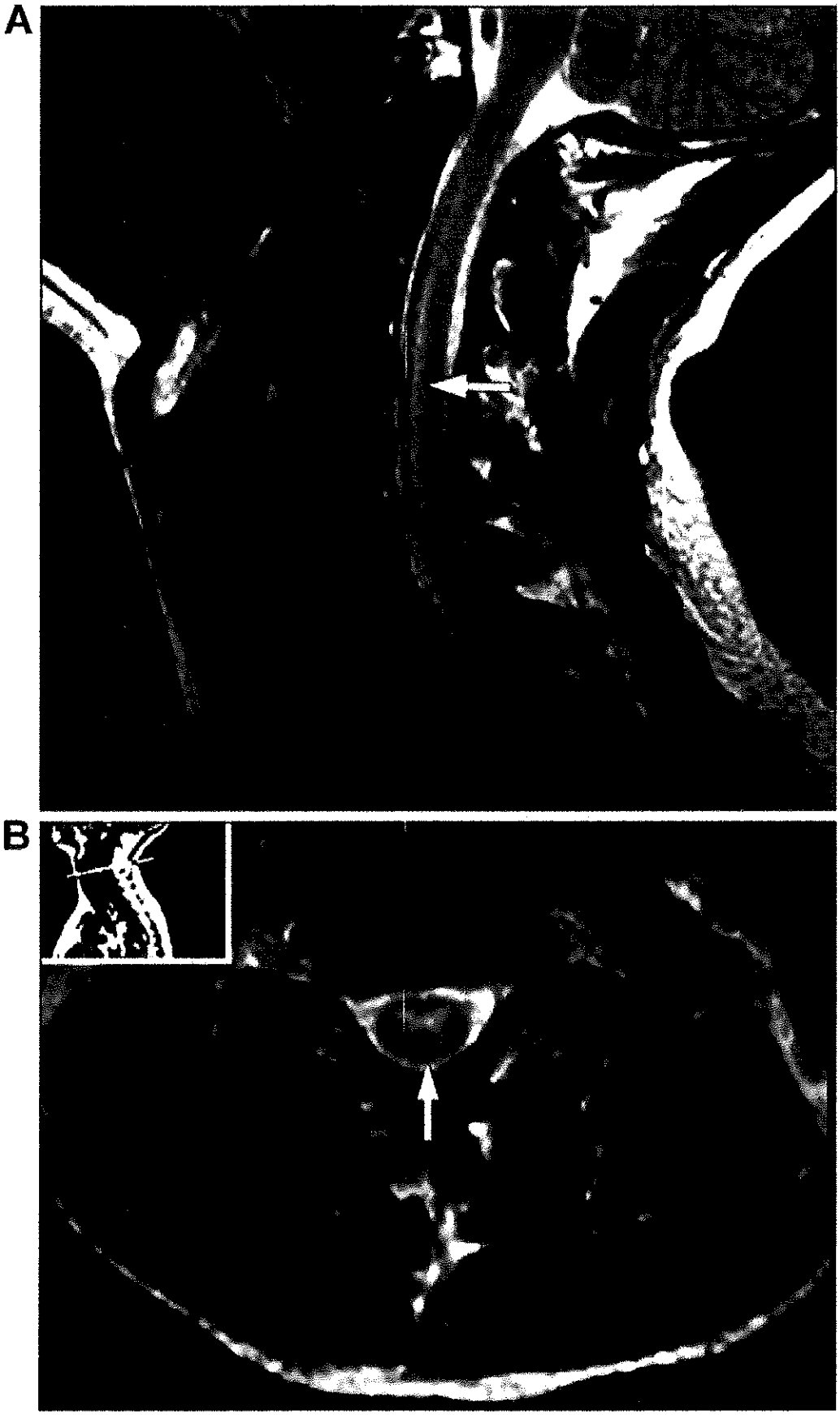


FIGURE 3

MRI of a suspected EV-D68 AFM patient. The MRI presents sagittal **(A,C)** and axial images **(B,D)** of the central nerves system. **(A)** Presents a case where the whole central gray matter was involved, producing a characteristic "H" pattern on

axial image **(B)**. **(C)** Presents a case where T2 hyperintensity was confined to the left anterior horn cells, which is demonstrated on the axial image **(D)**. Taken from Maloney et al., 2015. Order License Id: 4382500446364.


A
B

Open in a separate window

FIGURE 4

MRI of a poliovirus AFM patient. The MRI presents a sagittal **(A)** and an axial image **(B)** of the central nerves system. **(A)** presents a case showing hyperintensities involving the anterior horn cells from C3 to C7. **(B)**demonstrates the same case as an axial image. Taken from Haq and Wasay (2006). Order License Id: 4382500944212.

Treatment Options

Increasing reports of enhanced pathogenicity and the severity of the paralysis in the affected individuals have led to urgency in finding an effective treatment for EV-D68. At present, there is no vaccine or therapy which exists against EV-D68. A study carried out by Rhoden et al. (2015) evaluated several anti-viral candidates for EV-D68, with some promising results; V-7404, a protease inhibitor currently in development to treat poliovirus in immunodeficient patients; DAS181, similarly in development however to treat Influenza and Parainfluenza virus infections, and finally Rupintrivir, another protease inhibitor, not currently being developed further. Both V-7404 and Rupintrivir were able to inhibit all four tested EV-D68 strains (one Fermon and three 2014 outbreak strains). DAS181, which works as a sialidase was comparable in effectiveness, with a slightly EC50. However, these results were only obtained from *in vitro* testing, and clinical studies with these agents will not be available in the intermediate future.

A further study (Tyler, 2015) investigated the use of an already FDA approved drug, Fluoxetine, a serotonin inhibitor normally used as an antidepressant. The study found Fluoxetine inhibited the replication of EV-D68 in HeLa cells by a direct interaction with the 2C protein, which is thought to have a function in assembly. It is unknown if the tested strain was from the 2014 outbreak. However, Fluoxetine administration was noted to result in low maximal plasma levels, which could lead to problems in *in vivo*investigations (Rhoden et al., 2015).

As EV-D68 is a relatively emerging pathogen in terms of increased pathogenicity in recent years, treatment options are still far off. Exploring existing treatments for viruses, other than EV-D68, which present with similar symptoms, is therefore an attractive strategy (CDC and NCIRD, 2014). Recently, investigations were carried out (Hixon et al., 2017a) into three different empiric therapies that could decrease the severity of paralysis in a mouse model. The mice were intramuscularly injected with one of the 2014 outbreak strains. Positive results were obtained with hIVIG, which reduced paralysis and spinal cord viral loads. Fluoxetine was shown to have

a neutral effect, contrasting to Tyler, 2015 and dexamethasone worsened outcomes for the mice, causing increased mortality, possibly due to reducing the immune response and increasing viral replication. Disadvantageous results of corticosteroid treatment were also seen when these agents were used in an outbreak of neuro-invasive EV-A71 in Cambodia (World Health Organization [WHO], 2012). So far this research presents promising results for hIVIG therapy (pre and early post-infection) by providing potential protection against one of the most severe manifestations of an EV-D68 infection. Indeed, hIVIG therapy has been used previously and has arguably been successful in a least one suspected AFM case associated with WNV (Walid and Mahmoud, 2009). However, a further study (Chong et al., 2018) found hIVIG given to patients initially after onset of neurological symptoms, led to a poor prognosis.

As permanent functional impairment appears to be common in EV-D68 associated AFM (Messacar et al., 2016), solutions are also being sought to improve the outcome of patients after the paralysis has become irreversible. A potentially promising technique is nerve and muscle transfer, which was historically used for poliomyelitis associated paralysis (Messacar et al., 2016). As such, one study (Saltzman et al., 2016) describes the results of a trial involving nerve transfers in several patients following AFM associated EV-D68. In most of the cases proximal nerves were transferred, with one case undergoing bilateral nerve transfers. The study showed promising results with some muscle strength regained over a 6-month follow-up.

Non-polio Enterovirus Awareness and Its Subsequent Increased Surveillance

In the United States, published results from various surveillance studies are presented through the CDC. However, EV-D68 is only voluntarily reported in most states in the United States and is not a national notifiable disease (CDC and NNDSS, 2018). Similarly, the ECDC does not have an active surveillance system in place and relies on member states to provide updates on circulation of various EV. Therefore non-polio enterovirus surveillance in Europe can also depend on local interests of specific laboratories and national health institutes. As it is not mandatory to report EV-D68 in Europe (except Norway), and just in a few states in the United States, little real-time information is known on the exact numbers. This is a current challenge for EV-D68 data. However this may change now that the PAHO/WHO has introduced the recommendation of including respiratory samples in suspected EV-D68 cases (PAHO/WHO report, 2017).

Several surveillance systems are in place throughout Europe. One example is the enhanced non-polio EV surveillance system implemented in Denmark in which

respiratory samples were included, along with clinical description and genetic characterisation of the viruses (Barnadas et al., 2017). Similarly, in collaboration with the Dutch National Institute of Health, the TYPENED initiative [Typing network Netherlands] is involved in sequencing and collection of data (Niesters et al., 2013). In France, two EV National Reference Laboratories (in Clermont-Ferrand and Lyon) report the number of enterovirus infections and type of samples analyzed on a regular basis.[1] (Schuffenecker et al., 2016). Additionally, networks such as ENPEN exist to increase knowledge and communicate both epidemiological and clinical data during outbreaks, and to chart emerging infections. Such networks have been instrumental in raising awareness of EV-D68 and other non-polio EV through an email alert system and conferences (Harvala et al., 2018).

Go to:

Discussion

Burden of Disease and the Problems of Underdiagnosing

Enterovirus D68 made headlines worldwide in 2014 as a mysterious, relatively unknown virus was capable of causing severe illness. The CDC currently monitors and provides monthly updates on the incidence of AFM, which subsequently revealed another upsurge in 2016. Since no recent data has been published on the number of confirmed EV-D68 infections or the number of AFM cases in Europe, more testing and communication is required to understand both the EV-D68 infection patterns and the frequency at which this virus causes AFM. Specific EV-D68 assays have been developed to rapidly diagnose infections. Despite the existing evidence for the association between EV-D68 and AFM (Hixon et al., 2017b; Messacar et al., 2018), EV-D68 is overlooked due to insufficient knowledge, sampling, laboratory testing and communication between healthcare professionals and little surveillance from public health authorities.

Future Perspective and Directions

The current surveillance systems for poliovirus such as GPEI, environmental and AFP surveillance have been instrumental in nearly eradicating polio. Integrating EV-D68 into these established surveillance systems, would be highly effective in understanding the true burden of disease and prepare hospitals and laboratories for upcoming outbreaks. NGS has the potential to be a powerful tool in investigating emerging and untypeable pathogens. It could also be used to understand the host-pathogen relationship during an infection and to understand the evolution of these

viruses. However, there are still both technical and financial obstacles left to overcome before its used in routine practice (Rutvisuttinunt et al., 2017).

Further outbreaks of EV-D68 can be expected and could subsequently lead to an increase in EV-D68 associated AFM cases. EV-D68 numbers and AFM cases have increased in autumn of 2018. This has been communicated by the EV-D68 network made up of virologists and clinicians who collaborated during the 2014 and 2016 outbreaks. This has included two recent AFM cases from the Netherlands. Preliminary typing results obtained from the University Medical Center Groningen, the Netherlands, have indicated that recent EV-D68 samples have clustered into the B3 and A2 subclades. It is imperative to have effective and streamlined diagnostic procedures along with stewardship models to deal with the potential increase in cases. As a result, awareness needs to be created targeting clinicians and hospital wards in order to make clinical staff are aware of the virus. Continued interdisciplinary communication is important to ensure EV-D68 is translated across each medical field appropriately.

As effective treatment of EV-D68 infections and AFM are thus far unsubstantial, the focus may need to be placed on the development of a vaccine. Currently efficient vaccines are available for a few members of the enterovirus genus i.e., poliovirus and EV-A71 (Klein and Chong, 2015). These viruses however, have shown high incidences therefore it could be questioned whether a vaccine should be developed for EV-D68 at this time for economic reasons. Yet these vaccines have transformed the fight against these fatal EV. Using current knowledge and approaches, development of a vaccine against EV-D68 is technically achievable. A group recently published the results of a trial involving insect cell-expressed EV-D68 virus-like particle as a promising candidate for an EV-D68 vaccine (Dai et al., 2018). At present, the number of infections and life-threating cases do not reflect those of poliovirus, possibly due to lack of reporting with the current voluntary reporting systems. Only increased surveillance and diagnosis will make it possible to expose the extent of the EV-D68 threat.

Go to:

Author Contributions

HC drafted the manuscript. RP, MK, CVL-B, and HN revised the work critically and gave final approval before publication.

Go to:

Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Go to:

Abbreviations

AFM	acute flaccid myelitis
AFP	acute flaccid paralysis
CDC	Centers for Disease Control and Prevention
CNS	central nerve system
CSF	cerebral spinal fluid
EC50	lower effective concentration
ECDC	European Centre for Disease Prevention and Control
ENPEN	European Non-Polio Enterovirus Network
ESCV	European Society of Clinical Virology
EV	enteroviruses

EV-A71	enterovirus A71
EV-D68	enterovirus D68
FDA	Food and Drug Administration
GBS	Guillain-Barré syndrome
GPEI	Global Polio Eradication Initiative
hIVIG	human intravenous immunoglobulin
ICAM-5	neuron-expressed intercellular adhesion molecule 5
IP	intraperitoneally
MRI	magnetic resonance imaging
NCIRD	The National Center for Immunization and Respiratory Diseases
NGS	next generation sequencing

NNDSS	National Notifiable Diseases Surveillance System
PAHO	Pan American Health Organization
RNA	ribonucleic acid
RT-PCR	reverse transcriptase PCR
USA	United States
UTR	untranslated region
VP1	viral protein 1
VP4-2	Complete viral protein 4 and partial viral protein 2
WHO	World Health Organization
WNV	West Nile virus

Funding. HC has received funding from the European Union's Horizon 2020 research and innovation program 493, under the Marie Sklodowska-Curie grant agreement 713660 (MSCA-COFUND-201-DP 494 "Pronkjewail").

[1]http://cnr.chu-clermontferrand.fr/CNR/

Go to:

References

1. Aliabadi N., Messacar K., Pastula D., Robinson C., Leshem E., Sejvar J., et al. (2016). Enterovirus D68 infection in children with acute flaccid Myelitis, Colorado, USA, 2014. *Emerg. Infect. Dis.* 221387–1394. 10.3201/eid2208.151949 [PMC free article] [PubMed] [CrossRef]
2. Antona D., Kossorotoff M., Schuffenecker I., Mirand A., Leruez-Ville M., Bassi C., et al. (2016). Severe paediatric conditions linked with EV-A71 and EV-D68, France, May to October 2016. *Euro Surveill.* 21:30402. 10.2807/1560-7917.ES.2016.21.46.30402 [PMC free article] [PubMed] [CrossRef]
3. Baggen J., Thibaut H., Strating J., Van Kuppeveld F. (2018). The life cycle of non-polio enteroviruses and how to target it. *Nat. Rev. Microbiol.* 16 368–381. 10.1038/s41579-018-0005-4 [PubMed] [CrossRef]
4. Barnadas C., Midgley S., Skov M., Jensen L., Poulsen M., Fischer T. (2017). An enhanced *Enterovirus* surveillance system allows identification and characterization of rare and emerging respiratory enteroviruses in Denmark, 2015– 16. *J. Clin. Virol.* 93 40–44. 10.1016/j.jcv.2017.05.017 [PubMed] [CrossRef]
5. Blomqvist S., Savolainen C., Råman L., Roivainen M., Hovi T. (2002). Human rhinovirus 87 and enterovirus 68 represent a unique serotype with rhinovirus and enterovirus features. *J. Clin. Microbiol.* 40 4218–4223. 10.1128/JCM.40.11.4218-4223.2002 [PMC free article] [PubMed] [CrossRef]
6. Cabrerizo M., García-Iñiguez J. P., Munell F., Madurga-Revilla P., Rodrigo C., Martínez-Sapiña A., et al. (2017). First cases of severe flaccid paralysis associated with enterovirus D68 infection in Spain, 2015–2016. *Pediatr. Infect. Dis. J.* 36 1214–1216. 10.1097/INF.0000000000001668 [PubMed] [CrossRef]
7. CDC and NCIRD (2018). *Acute Flaccid Myelitis Investigation.* Available at: https://www.cdc.gov/acute-flaccid-myelitis/afm-surveillance.html [accessed July 03, 2018]
8. CDC and NCIRD (2014). *Acute Flaccid Myelitis: Interim Considerations for Clinical Management.* Available at: https://www.cdc.gov/acute-flaccid-myelitis/hcp/clinical-management.html [accessed July 03, 2018]
9. CDC and NCIRD (2015). *For Clinicians: Diagnosing Acute Flaccid Myelitis (AFM) in the United States.* Available

at: https://www.cdc.gov/acute-flaccid-myelitis/downloads/afm-presentation.pdf[accessed July 03, 2018]

10. CDC and NNDSS (2018). *National Notifiable Conditions.* Available at: https://wwwn.cdc.gov/nndss/conditions/notifiable/2018/ [accessed April 16, 2018].
11. Chong P., Kira R., Mori H., Okumura A., Torisu H., Yasumoto S., et al. (2018). Clinical features of acute flaccid myelitis temporally associated with an enterovirus D68 outbreak: results of a nationwide survey of acute flaccid paralysis in Japan, August-December 2015. *Clin. Infect. Dis.* 66653–664. 10.1093/cid/cix860 [PMC free article] [PubMed] [CrossRef]
12. Craighead J. (ed.) (2000). "Enteroviruses," in *Pathology and Pathogenesis of Human Viral Disease*(Amsterdam: Elsevier;) 1–28. 10.1016/B978-012195160-3/50002-9 [CrossRef]
13. Dai W., Zhang C., Zhang X., Xiong P., Liu Q., Gong S., et al. (2018). A virus-like particle vaccine confers protection against enterovirus D68 lethal challenge in mice. *Vaccine* 36 653–659. 10.1016/j.vaccine.2017.12.057 [PubMed] [CrossRef]
14. Diaz-Decaro J., Green N., Godwin H. (2018). Critical evaluation of FDA-approved respiratory multiplex assays for public health surveillance. *Expert Rev. Mol. Diagn.* 18 631–643. 10.1080/14737159.2018.1487294 [PubMed] [CrossRef]
15. Dik J.-W., Poelman R., Friedrich A., Panday P., Lo-Ten-Foe J., Van Assen S., et al. (2016). An integrated stewardship model: antimicrobial, infection prevention and diagnostic (AID). *Future Microbiol.* 11 93–102. 10.2217/fmb.15.99 [PubMed] [CrossRef]
16. Duong V., Mey C., Eloit M., Zhu H., Danet L., Huang Z., et al. (2016). Molecular epidemiology of human enterovirus 71 at the origin of an epidemic of fatal hand, foot and mouth disease cases in Cambodia. *Emerg. Microbes. Infect.* 5:e104. 10.1038/emi.2016.101 [PMC free article] [PubMed] [CrossRef]
17. Dyda A., Stelzer-Braid S., Adam D., Chughtai A., Raina MacIntyre C., Amalie D., et al. (2018). The association between acute flaccid myelitis (AFM) and *Enterovirus* D68 (EV-D68) – what is the evidence for causation? *Euro Surveill.* 23:17-00310. 10.2807/1560-7917.ES.2018.23.3.17-00310[PMC free article] [PubMed] [CrossRef]
18. Dyrdak R., Grabbe M., Hammas B., Ekwall J., Hansson K. E., Luthander J., et al. (2016). Outbreak of enterovirus D68 of the new B3 lineage in Stockholm, Sweden, August to September 2016. *Euro Surveill.* 21:30403. 10.2807/1560-7917.ES.2016.21.46.30403 [PMC free article] [PubMed] [CrossRef]

19. ECDC. (2016). *Rapid Risk Assessment – Enterovirus Detections Associated with Severe Neurological Symptoms in Children and Adults in European Countries.* Solna Municipality: ECDC.
20. Escalona M., Rocha S., Posada D. (2016). A comparison of tools for the simulation of genomic next-generation sequencing data. *Nat. Rev. Genet.* 17 459–469. 10.1038/nrg.2016.57 [PMC free article][PubMed] [CrossRef]
21. Esposito S., Bosis S., Niesters H., Principi N. (2015). Enterovirus D68 infection. *Viruses* 7 6043–6050. 10.3390/v7112925 [PMC free article] [PubMed] [CrossRef]
22. Esposito S., Chidini G., Cinnante C., Napolitano L., Giannini A., Terranova L., et al. (2017). Acute flaccid myelitis associated with enterovirus-D68 infection in an otherwise healthy child. *Virol. J.*14:4. 10.1186/s12985-016-0678-0 [PMC free article] [PubMed] [CrossRef]
23. Esposito S., Lunghi G., Zampiero A., Tagliabue C., Orlandi A., Torresani E., et al. (2016). Enterovirus-D68 in the cerebrospinal fluid of two children with aseptic meningitis. *Pediatr. Infect. Dis. J.* 35 589–591. 10.1097/INF.0000000000001085 [PubMed] [CrossRef]
24. Foster C. B., Friedman N., Carl J., Piedimonte G. (2015). Enterovirus D68: a clinically important respiratory enterovirus. *Cleve. Clin. J. Med.* 82 26–31. 10.3949/ccjm.82a.14166 [PubMed] [CrossRef]
25. Giombini E., Rueca M., Barberi W., Iori A. P., Castilletti C., Scognamiglio P., et al. (2017). Enterovirus D68–associated acute flaccid myeltis in immunocompromised women in Italy. *Emerg. Infect. Dis.* 23 1690–1693. 10.3201/eid2310.170792 [PMC free article] [PubMed] [CrossRef]
26. Gong Y.-N., Yang S.-L., Shih S.-R., Huang Y.-C., Chang P.-Y., Huang C.-G., et al. (2016). Molecular evolution and the global reemergence of enterovirus D68 by genome-wide analysis. *Medicine*95:e4416. 10.1097/MD.0000000000004416 [PMC free article] [PubMed] [CrossRef]
27. Greninger A., Naccache S., Messacar K., Clayton A., Yu G., Somasekar S., et al. (2015). A novel outbreak enterovirus D68 strain associated with acute flaccid myelitis cases in the USA (2012-14): a retrospective cohort study. *Lancet Infect. Dis.* 15 671–682. 10.1016/S1473-3099(15)70093-9[PMC free article] [PubMed] [CrossRef]
28. Haq A., Wasay M. (2006). Magnetic resonance imaging in poliomyelitis. *JAMA Neurol.* 63:778. 10.1001/archneur.63.5.778 [PubMed] [CrossRef]
29. Harvala H., Broberg E., Benschop K., Berginc N., Ladhani S., Susi P., et al. (2018). Recommendations for enterovirus diagnostics and characterisation

within and beyond Europe. *J. Clin. Virol.* 101 11–17. 10.1016/j.jcv.2018.01.008 [PubMed] [CrossRef]

30. Hixon A., Clarke P., Tyler K. (2017a). Evaluating treatment efficacy in a mouse model of enterovirus D68-associated paralytic myelitis. *J. Infect. Dis.* 216 1245–1253. 10.1093/infdis/jix468[PMC free article] [PubMed] [CrossRef]
31. Hixon A., Yu G., Leser J., Yagi S., Clarke P., Chiu C., et al. (2017b). A mouse model of paralytic myelitis caused by enterovirus D68. *PLoS Pathogens* 13:e1006199. 10.1371/journal.ppat.1006199[PMC free article] [PubMed] [CrossRef]
32. Holm-Hansen C., Midgley S., Fischer T. (2016). Global emergence of enterovirus D68: a systematic review. *Lancet Infect. Dis.* 16 e64–e75. 10.1016/S1473-3099(15)00543-5 [PubMed] [CrossRef]
33. Huang H., Shih S. (2015). Neurotropic enterovirus infections in the central nervous system. *Viruses* 76051–6066. 10.3390/v7112920 [PMC free article] [PubMed] [CrossRef]
34. Huang W., Wang G., Zhuge J., Nolan S., Dimitrova N., Fallon J. (2015). Whole-Genome sequence analysis reveals the enterovirus D68 isolates during the United States 2014 outbreak mainly belong to a novel clade. *Sci. Rep.* 5:15223. 10.1038/srep15223 [PMC free article] [PubMed] [CrossRef]
35. Imamura T., Okamoto M., Nakakita S.-I., Suzuki A., Saito M., Tamaki R., et al. (2014). Antigenic and receptor binding properties of enterovirus. *J. Virol.* 88 2374–2384. 10.1128/JVI.03070-13[PMC free article] [PubMed] [CrossRef]
36. Imamura T., Oshitani H. (2015). Global reemergence of enterovirus D68 as an important pathogen for acute respiratory infections. *Rev. Med. Virol.* 25 102–114. 10.1002/rmv.1820 [PMC free article][PubMed] [CrossRef]
37. Jasti A., Selmi C., Sarmiento-Monroy J., Vega D., Anaya J., Gershwin M. (2016). Guillain-Barré syndrome: causes, immunopathogenic mechanisms and treatment. *Expert Rev. Clin. Immunol.* 121175–1189. 10.1080/1744666X.2016.1193006 [PubMed] [CrossRef]
38. Kaida A., Iritani N., Yamamoto S., Kanbayashi D., Hirai Y., Togawa M., et al. (2017). Distinct genetic clades of enterovirus D68 detected in 2010 2013 and 2015 in Osaka City, Japan. *PLoS One*12:e0184335. 10.1371/journal.pone.0184335 [PMC free article] [PubMed] [CrossRef]
39. Kirolos A., Mark K., Waugh C., Shetty J., McCallum A., Templeton K., et al. (2017). Cluster of acute flaccid paralysis in children following

enterovirus D68 infection in Scotland. *Eur. J. Public Health.* 27:ckx187.697 10.1093/eurpub/ckx187.697 [CrossRef]

40. Klein M., Chong P. (2015). Is a multivalent hand, foot, and mouth disease vaccine feasible? *Hum. Vaccin. Immunother.* 11 2688–2704. 10.1080/21645515.2015.1049780 [PMC free article] [PubMed] [CrossRef]
41. Knoester M., Helfferich J., Poelman R., Van Leer-Buter C., Brouwer O., Niesters H. (2018). Twenty-Nine cases of enterovirus-D68 associated acute flaccid myelitis in Europe 2016; A case series and epidemiologic overview. *Pediatr. Infect. Dis. J.* 10.1097/INF.0000000000002188 [Epub ahead of print]. [PubMed] [CrossRef]
42. Knoester M., Schölvinck E., Poelman R., Smit S., Vermont C., Niesters H., et al. (2017). Upsurge of enterovirus D68 the Netherlands, 2016. *Emerg. Infect. Dis.* 23 140–143. 10.3201/eid2301.161313[PMC free article] [PubMed] [CrossRef]
43. Kreuter J. D., Barnes A., McCarthy J. E., Schwartzman J. D., Oberste M. S., Rhodes C. H., et al. (2011). A fatal central nervous system enterovirus 68 infection. *Arch. Pathol. Lab. Med.* 135 793–796. 10.1043/2010-0174-CR.1 [PubMed] [CrossRef]
44. Lang M., Mirand A., Savy N., Henquell C., Maridet S., Perignon R., et al. (2014). Acute flaccid paralysis following enterovirus D68 associated pneumonia, France, 2014. *Euro Surveill.* 19:2095210.2807/1560-7917.ES2014.19.44.20952 [PubMed] [CrossRef]
45. Levy A., Roberts J., Lang J., Tempone S., Kesson A., Dofai A., et al. (2015). Enterovirus D68 disease and molecular epidemiology in Australia. *J. Clin. Virol.* 69 117–121. 10.1016/j.jcv.2015.06.079 [PubMed] [CrossRef]
46. Maloney J., Mirsky D., Messacar K., Dominguez S., Schreiner T., Stence N. (2015). MRI findings in children with acute flaccid paralysis and cranial nerve dysfunction occurring during the 2014 enterovirus D68 outbreak. *Am. J. Neuroradiol.* 36 245–250. 10.3174/ajnr.A4188 [PubMed] [CrossRef]
47. Martin G., Li R., Cook V., Carwana M., Tilley P., Sauve L., et al. (2016). Respiratory presentation of pediatric patients in the 2014 enterovirus D68 outbreak. *Can. Respir. J.* 2016:8302179. 10.1155/2016/8302179 [PMC free article] [PubMed] [CrossRef]
48. McAllister S., Schleiss M., Arbefeville S., Steiner M., Hanson R., Pollock C., et al. (2015). Epidemic 2014 enterovirus D68 cross-reacts with human rhinovirus on a respiratory molecular diagnostic platform. *PLoS One* 10:e0118529. 10.1371/journal.pone.0118529 [PMC free article] [PubMed] [CrossRef]
49. Messacar K., Asturias E., Hixon A., Van Leer-Buter C. M., Niesters H., Tyler K., et al. (2018). Personal view enterovirus D68 and acute flaccid

myelitis—evaluating the evidence for causality.*Lancet Infect. Dis.* 18 e239–e247. 10.1016/S1473-3099(18)30094-X [PubMed] [CrossRef]

50. Messacar K., Schreiner T., Van Haren K., Yang M., Glaser C., Tyler K., et al. (2016). Acute flaccid myelitis: a clinical review of US cases 2012–2015. *Ann. Neurol.* 80 326–338. 10.1002/ana.24730[PMC free article] [PubMed] [CrossRef]
51. Midgley C., Watson J., Nix W., Curns A., Rogers S., Brown B., et al. (2015). Severe respiratory illness associated with a nationwide outbreak of enterovirus D68 in the USA (2014): a descriptive epidemiological investigation. *Lancet Respir. Med.* 3 879–887. 10.1016/S2213-2600(15)00335-5[PMC free article] [PubMed] [CrossRef]
52. Morrey J., Wang H., Hurst B., Zukor K., Siddharthan V., Van Wettere A., et al. (2018). Causation of acute flaccid paralysis by myelitis and myositis in enterovirus-D68 infected mice deficient in interferon αβ/γ receptor deficient mice. *Viruses* 10:E33. 10.3390/v10010033 [PMC free article][PubMed] [CrossRef]
53. National Center for Immunization and Respiratory Diseases [Ncird], Division of Viral Diseases (2017). *Enterovirus D68.* Available at: https://www.cdc.gov/non-polio-enterovirus/about/ev-d68.html[accessed April 11, 2018]
54. Niesters H., Rossen J., van der Avoort H., Baas D., Benschop K., Claas E., et al. (2013). Laboratory-based surveillance in the molecular era: the TYPENED model, a joint data-sharing platform for clinical and public health laboratories. *Euro Surveill.* 18:20387. 10.2807/ese.18.04.20387-en [PubMed] [CrossRef]
55. Nix A. W., Oberste M., Pallansch M. (2006). Sensitive, seminested PCR amplification of VP1 sequences for direct identification of all enterovirus serotypes from original clinical specimens. *J. Clin. Microbiol.* 44 2698–2704. 10.1128/JCM.00542-06 [PMC free article] [PubMed] [CrossRef]
56. Noor A., Krilov L. (2016). Enterovirus infections. *Pediatr. Rev.* 37 505–515. 10.1542/pir.2016-0103 [PubMed] [CrossRef]
57. Ohka S., Yang W.-X., Terada E., Iwasaki K., Nomoto A. (1998). Retrograde transport of intact poliovirus through the axon via the fast transport system. *Virology* 250 67–75. 10.1006/viro.1998.9360 [PubMed] [CrossRef]
58. PAHO/WHO. (2016). *Report From: The Argentina International Health Regulations National Focal Point.* Washington, DC: PAHO.
59. PAHO/WHO report. (2017). *Epidemiological Alert Acute Flaccid Myelitis Associated with Enterovirus D68 in the Context of Acute Flaccid Paralysis Surveillance.* Washington, DC: PAHO.

60. Paul J. (1971). *A History of Poliomyelitis.* New Haven, CT: Yale University Press.
61. Pfeiffer H., Bragstad K., Skram M., Dahl H., Knudsen P., Chawla M., et al. (2015). Two cases of acute severe flaccid myelitis associated with enterovirus D68 infection in children, Norway, autumn 2014. *Euro Surveill.* 20:21062. 10.2807/1560-7917.ES2015.20.10.21062 [PubMed] [CrossRef]
62. Poelman R., Schölvinck E., Borger R., Niesters H., Van Leer-Buter C. (2015a). The emergence of enterovirus D68 in a Dutch University Medical Center and the necessity for routinely screening for respiratory viruses. *J. Clin. Virol.* 62 1–5. 10.1016/j.jcv.2014.11.011 [PubMed] [CrossRef]
63. Poelman R., Schuffenecker I., Van Leer-Buter C., Josset L., Niesters H., Lina B., et al. (2015b). European surveillance for enterovirus D68 during the emerging North-American outbreak in 2014. *J. Clin. Virol.* 71 1–9. 10.1016/j.jcv.2015.07.296 [PubMed] [CrossRef]
64. Poritz M., Blaschke A., Byington C., Meyers L., Nilsson K., Jones D., et al. (2011). Filmarray, an automated nested multiplex PCR system for multi-pathogen detection: development and application to respiratory tract infection. *PLoS One* 6:e26047. 10.1371/journal.pone.0026047 [PMC free article][PubMed] [CrossRef]
65. Rahamat-Langendoen J., Riezebos-Brilman A., Borger R., van der Heide R., Brandenburg A., Schölvinck E., et al. (2011). Upsurge of human enterovirus 68 infections in patients with severe respiratory tract infections. *J. Clin. Virol.* 52 103–106. 10.1016/j.jcv.2011.06.019 [PubMed] [CrossRef]
66. Rhoden E., Zhang M., Nix W., Oberste M. (2015). In vitro efficacy of antiviral compounds against enterovirus D68. *Antimicrob. Agents Chemother.* 59 7779–7781. 10.1128/AAC.00766-15[PMC free article] [PubMed] [CrossRef]
67. Ruggieri V., Paz M. I., Peretti M. G., Rugilo C., Bologna R., Freire C., et al. (2017). Enterovirus D68 infection in a cluster of children with acute flaccid myelitis, Buenos Aires, Argentina, 2016. *Eur. J. Paediatr. Neurol.* 21 884–890. 10.1016/j.ejpn.2017.07.008 [PubMed] [CrossRef]
68. Rutvisuttinunt W., Klungthong C., Thaisomboonsuk B., Chinnawirotpisan P., Ajariyakhajorn C., Manasatienkij W., et al. (2017). Retrospective use of next-generation sequencing reveals the presence of Enteroviruses in acute influenza-like illness respiratory samples collected in South/South-East Asia during 2010–2013. *J. Clin. Virol.* 94 91–99. 10.1016/j.jcv.2017.07.004 [PubMed] [CrossRef]

69. Saltzman E., Vu A., Schwenker A., Raney S., Sneag D., Yakuboff K., et al. (2016). Nerve transfers for enterovirus D68-induced acute flaccid myelitis. *HAND* 11:97S. 10.1016/j.pediatrneurol.2018.07.018 [CrossRef]
70. Schuffenecker I., Mirand A., Josset L., Henquell C., Hecquet D., Pilorgé L., et al. (2016). Epidemiological and clinical characteristics of patients infected with enterovirus D68 France, July to December 2014. *Euro Surveill.* 21:30226. 10.2807/1560-7917.ES.2016.21.19.30226 [PubMed] [CrossRef]
71. Sejvar J., Lopez A., Cortese M., Leshem E., Pastula D., Miller L., et al. (2016). Acute flaccid myelitis in the United States, August-December 2014: results of nationwide surveillance. *Clin. Infect. Dis.* 63 737–745. 10.1093/cid/ciw372 [PMC free article] [PubMed] [CrossRef]
72. Shibib D., Matushek S., Beavis K., Gawel S., Charnot-Katsikas A. (2016). BioFire film array respiratory panel for detection of enterovirus D68. *J. Clin. Microbiol.* 54 457–459. 10.1128/JCM.02339-15 [PMC free article] [PubMed] [CrossRef]
73. Stacpoole S., Molyneux A., Bäumer D. (2017). Acute segmental poliomyelitis-like flaccid paralysis in an adult in the UK, associated with enterovirus D68. *Pract. Neurol.* 17 297–301. 10.1136/practneurol-2017-001609 [PubMed] [CrossRef]
74. The Global Poliovirus Eradication Initiative [GPEI] (2018). *Global Polio Eradication Initiative.* Available at http://polioeradication.org/where-we-work/polio-endemic-countries/ [accessed June 03, 2018] 10.1136/practneurol-2017-001609 [CrossRef]
75. Tokarz R., Firth C., Madhi S., Howie S., Wu W., Sall A., et al. (2012). Worldwide emergence of multiple clades of enterovirus 68. *J. Gen. Virol.* 93(Pt 9) 1952–1958. 10.1099/vir.0.043935-0[PMC free article] [PubMed] [CrossRef]
76. Tse M., Lane C., Mott K., Onlamoon N., Hsiao H., Perng G. (2009). ICAM-5 modulates cytokine/chemokine production in the CNS during the course of herpes simplex virus type 1 infection. *J. Neuroimmunol.* 213 12–19. 10.1016/j.jneuroim.2009.06.007 [PMC free article][PubMed] [CrossRef]
77. Tyler K. (2015). Rationale for the evaluation of fluoxetine in the treatment of enterovirus d68-associated acute flaccid myelitis. *JAMA Neurol.* 72 493–494. 10.1001/jamaneurol.2014.4625[PMC free article] [PubMed] [CrossRef]
78. Varghese R., Iyer A., Hunter K., Cargill J., Cooke R. (2015). Sampling the upper respiratory tract for enteroviral infection is important in the investigation of an acute neurological illness in children. *Eur. J. Paediatr. Neurol.* 19 494–495. 10.1016/j.ejpn.2015.03.009 [PubMed] [CrossRef]

79. Walid M., Mahmoud A. (2009). Successful treatment with intravenous immunoglobulin of acute flaccid paralysis caused by West Nile Virus. *Perm. J.* 13 43–46. 10.7812/TPP/09-028[PMC free article] [PubMed] [CrossRef]
80. Wang G., Zhuge J., Huang W., Nolan S., Gilrane V., Yin C., et al. (2017). Enterovirus D68 subclade B3 strain circulating and causing an outbreak in the United States in 2016. *Sci. Rep.* 7:1242. 10.1038/s41598-017-01349-4 [PMC free article] [PubMed] [CrossRef]
81. Washington State Department of Health (2016). *Communicable Disease Epidemiology. Acute Flaccid Myelitis and Poliomyelitis Reporting and Investigation Guideline.* Chesterfield: DCHS.
82. Wei W., Guo H., Chang J., Yu Y., Liu G., Zhang N., et al. (2016). ICAM-5 Telencephalin is a functional entry. *Cell Host Microbe* 20 631–641. 10.1016/j.chom.2016.09.013 [PubMed] [CrossRef]
83. Williams C., Thomas R., Pickersgill T., Lyons M., Lowe G., Stiff R., et al. (2016). Cluster of atypical adult Guillain-Barré syndrome temporally associated with neurological illness due to EV-D68 in children, South Wales, United Kingdom, October 2015 to January 2016. *Euro Surveill.* 21:30119. 10.2807/1560-7917.ES.2016.21.4.30119 [PubMed] [CrossRef]
84. World Health Organization [WHO] (2018). *Poliomyelitis.* Available at: http://www.who.int/mediacentre/factsheets/fs114/en/ [accessed April 11, 2018]
85. World Health Organization [WHO] (2012). *(Emergencies Preparedness, Response) Severe Complications of Hand, Foot and Mouth Disease (HFMD) Caused by EV-71 in Cambodia – Conclusion of the Joint Investigation.* Available at: http://www.who.int/csr/don/2012_07_13/en/. [accessed April 11, 2018]
86. Xiang Z., Xie Z., Liu L., Ren L., Xiao Y., Paranhos-Baccalà G., et al. (2016). Genetic divergence of enterovirus D68 in China and the United States. *Sci. Rep.* 6:27800. 10.1038/srep27800[PMC free article] [PubMed] [CrossRef]